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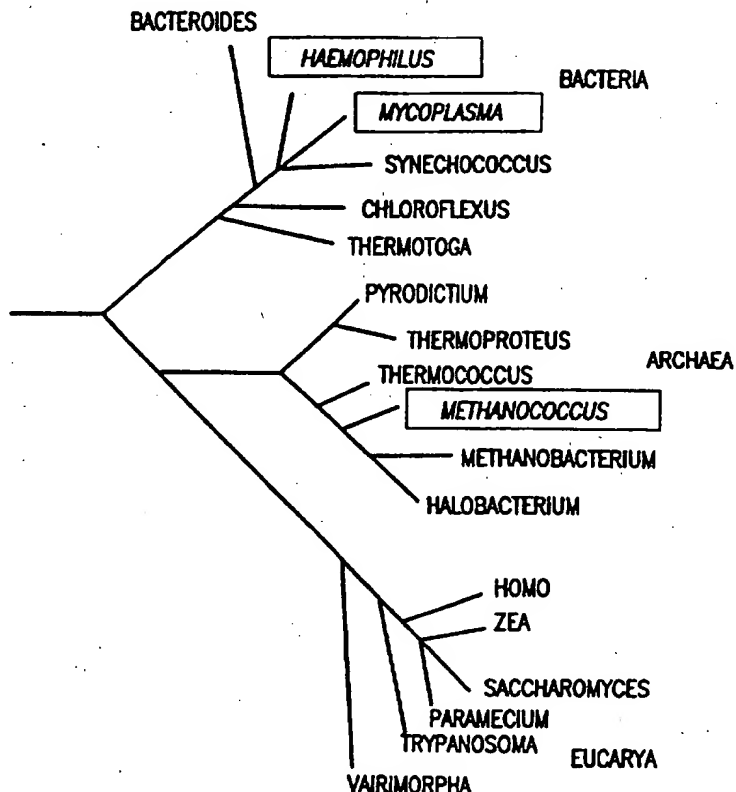
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## (57) Abstract

The present application describes the complete 1.66-megabase pair genome sequence of an autotrophic archaeon, *Methanococcus jannaschii*, and its 58- and 16-kilobase pair extrachromosomal elements. Also described are 1738 predicted protein-coding genes.



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# Complete Genome Sequence of the Methanogenic Archaeon, *Methanococcus jannaschii*

## *Background of the Invention*

### *Statement as to Rights to Inventions Made Under Federally-Sponsored Research and Development*

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government may have certain rights in the invention - DE-FC02-95ER61962; DE-FC02-95ER61963; and NAGW 2554.

### *Field of the Invention*

The present application discloses the complete 1.66-megabase pair genome sequence of an autotrophic archaeon, *Methanococcus jannaschii*, and its 58- and 16-kilobase pair extrachromosomal elements. Also identified are 1738 predicted protein-coding genes.

### *Related Background Art*

The view of evolution in which all cellular organisms are in the first instance either prokaryotic or eukaryotic was challenged in 1977 by the finding that on the molecular level life comprises three primary groupings (Fox, G.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:4537 (1977); Woese, C.R. & Fox, G.E., *Proc. Natl. Acad. Sci. USA* 74:5088 (1977); Woese, C.R., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:4576 (1990)): the eukaryotes (Eukarya) and two unrelated groups of prokaryotes, Bacteria and a new group now called the Archaea. Although Bacteria and Archaea are both prokaryotes in a cytological sense, they differ profoundly in their molecular makeup (Fox, G.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:4537 (1977); Woese, C.R. & Fox, G.E., *Proc. Natl. Acad. Sci. USA* 74:5088 (1977); Woese, C.R., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:4576 (1990)).

Several lines of molecular evidence even suggest a specific relationship between Archaea and Eukarya (Iwabe, N., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9355 (1989); Gogarten J.P., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:6661 (1989); Brown, J.R. and Doolittle, W.F., *Proc. Natl. Acad. Sci. USA* 92:2441 (1995)).

5           The era of true comparative genomics has been ushered in by complete genome sequencing and analysis. We recently described the first two complete bacterial genome sequences, those of *Haemophilus influenzae* and *Mycoplasma genitalium* (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)). Large scale DNA sequencing efforts also have  
10           produced an extensive collection of sequence data from eukaryotes, including *Homo sapiens* (Adams, M.D., *et al.*, *Nature* 377:3 (1995)) and *Saccharomyces cerevisiae* (Levy, J., *Yeast* 10:1689 (1994)).

*M. jannaschii* was originally isolated by J.A. Leigh from a sediment sample collected from the sea floor surface at the base of a 2600 m deep "white  
15           smoker" chimney located at 21° N on the East Pacific Rise (Jones, W., *et al.*, *Arch. Microbiol.* 136:254 (1983)). *M. jannaschii* grows at pressures of up to more than 500 atm and over a temperature range of 48-94 °C, with an optimum temperature near 85 °C (Jones, W., *et al.*, *Arch. Microbiol.* 136:254 (1983)). The organism is autotrophic and a strict anaerobe; and, as the name implies, it  
20           produces methane. The dearth of archaeal nucleotide sequence data has hampered attempts to begin constructing a comprehensive comparative evolutionary framework for assessing the molecular basis of the origin and diversification of cellular life.

### *Summary of the Invention*

25           The present invention is based on whole-genome random sequencing of an autotrophic archaeon, *Methanococcus jannaschii*. The *M. jannaschii* genome consists of three physically distinct elements: (i) a large circular chromosome; (ii) a large circular extrachromosomal element (ECE); and (iii) a small circular extrachromosomal element (ECE). The nucleotide sequences generated, the *M.*

*jannaschii* chromosome, the large ECE, and the small ECE, are respectively provided on pages 152-585 (SEQ ID NO:1), pages 585-600 (SEQ ID NO:2), and pages 601-605 (SEQ ID NO:3).

5 The present invention is further directed to isolated nucleic acid molecules comprising open reading frames (ORFs) encoding *M. jannaschii* proteins. The present invention also relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of *M. jannaschii* proteins. Further embodiments include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to the nucleotide sequence of a *M. jannaschii* ORF described herein.

10 The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, host cells containing the recombinant vectors, as well as methods for making such vectors and host cells for *M. jannaschii* protein production by recombinant techniques.

15 The invention further provides isolated polypeptides encoded by the *M. jannaschii* ORFs. It will be recognized that some amino acid sequences of the polypeptides described herein can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. In general, it is possible to replace residues which form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein.

20 In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope-bearing portion is an immunogenic or antigenic epitope useful for raising antibodies.

### Brief Description of the Figures

**Figure 1.** A schematic showing the relationship of the three domains of life based on sequence data from the small subunit of rRNA (Fox, G.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 74:4537 (1977); Woese, C.R. & Fox, G.E., *Proc. Natl. Acad. Sci. USA* 74:5088 (1977); Woese, C.R., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:4576 (1990)).

**Figure 2.** Structure of a putative family of insertion sequence (IS) elements in the *M. jannaschii* genome. The family of elements has been named ISAMJI and contains 11 members distributed among three groups (A, B, and C). The outer rectangle indicates the entire IS element; the interior rectangles indicate the predicted coding regions, oriented with the NH<sub>2</sub>-termini to the left. DNA immediately adjacent to the NH<sub>2</sub>-termini is 75 to 100% identical over 50 bp; DNA sequence similarity at the COOH-termini ends immediately after the stop codon. Black triangles indicate terminal inverted repeats. Fill patterns indicate which regions are missing from the elements in groups B and C. (A) Two copies of this family are 642 bp long and are 97% similar to each other at the nucleotide level. They appear to encode a protein 214 amino acids in length (ORFs MJ0017 and MJ1466) that are 27% identical to the IS240 transposase of *Bacillus thuriangiensis* (GenBank Accession number: M23741). (B) Eight copies of the family range in length from 358 to 360 bp and are missing a 342-bp internal region relative to the two members of group A. Some members of group B have putative frameshifts (indicated by solid arrows) and in-frame UGA codons (indicated by open arrows). (C) The single copy in group C is 265 bp in length and occurs on the large ECE. The 436 bp internal region missing from this element is different than that of the members of group B.

**Figure 3.** Structure of a multicopy repetitive element in the *M. jannaschii* genome. Of the 18 copies identified on the main chromosome, seven are oriented in one direction (plus strand) and 11 are oriented in the opposite strand. Each element consists of a long, 391- to 425-bp repeat segment (designated LR) followed by up to 25 short, 27- to 28-bp repeat segments (designated SR). Each

SR segment is separated by 31 to 51 bp of sequence that is unique within and between each complete repeat element. (A) The longest repeat element has an LR segment followed by 25 SR segments, and spans more than 2 kbp, and (B) the shortest complete element has an LR segment followed by two SR segments. (C) One element is present in the genome with five SR segments and no LR component. (D and E) The LR segments of two elements in the genome are truncated at the end adjacent to the SR segments, both are followed by a single SR segment.

Figure 4. Block diagram of a computer system 102 that can be used to implement the computer-based systems of present invention.

### *Detailed Description of the Invention*

The present invention is based on whole-genome random sequencing of an autotrophic archaeon, *Methanococcus jannaschii*. The *M. jannaschii* genome consists of three physically distinct elements: (i) a large circular chromosome of 1,664,976 base pairs (bp) (shown on pages 152-585 and in SEQ ID NO:1), which contains 1682 predicted protein-coding regions and has a G+C content of 31.4%; (ii) a large circular extrachromosomal element (the large ECE) of 58,407 bp (shown on pages 585-600 and in SEQ ID NO:2), which contains 44 predicted protein-coding regions and has a G+C content of 28.2%; and (iii) a small circular extrachromosomal element (the small ECE) of 16,550 bp (shown on pages 601-605 and in SEQ ID NO:3), which contains 12 predicted protein-coding regions and has a G+C content of 28.8%.

The primary nucleotide sequences generated, the *M. jannaschii* chromosome, the large ECE, and the small ECE, are provided in SEQ ID NOs:1, 2, and 3, respectively. As used herein, the "primary sequence" refers to the nucleotide sequence represented by the IUPAC nomenclature system. The present invention provides the nucleotide sequences of SEQ ID NOs:1, 2, and 3, or a representative fragment thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan.

As used herein, a "representative fragment" refers to *M. jannaschii* protein-encoding regions (also referred to herein as open reading frames), expression modulating fragments, uptake modulating fragments, and fragments that can be used to diagnose the presence of *M. jannaschii* in a sample. A non-limiting identification of such representative fragments is provided in Tables 2(a) and 3. As described in detail below, representative fragments of the present invention further include nucleic acid molecules having a nucleotide sequence at least 90% identical, preferably at least 95, 96%, 97%, 98%, or 99% identical, to an ORF identified in Table 2(a) or 3.

As indicated above, the nucleotide sequence information provided in SEQ ID NOs:1, 2 and 3 was obtained by sequencing the *M. jannaschii* genome using a megabase shotgun sequencing method. The sequences provided in SEQ ID NOs:1, 2 and 3 are highly accurate, although not necessarily a 100% perfect, representation of the nucleotide sequence of the *M. jannaschii* genome. As discussed in detail below, using the information provided in SEQ ID NOs:1, 2 and 3 and in Tables 2(a) and 3 together with routine cloning and sequencing methods, one of ordinary skill in the art would be able to clone and sequence all "representative fragments" of interest including open reading frames (ORFs) encoding a large variety of *M. jannaschii* proteins. In rare instances, this may reveal a nucleotide sequence error present in the nucleotide sequences disclosed in SEQ ID NOs: 1, 2, and 3. Thus, once the present invention is made available (i.e., once the information in SEQ ID NOs:1, 2, and 3 and in Tables 2(a) and 3 have been made available), resolving a rare sequencing error would be well within the skill of the art. Nucleotide sequence editing software is publicly available. For example, Applied Biosystem's (AB) AutoAssembler™ can be used as an aid during visual inspection of nucleotide sequences.

Even if all of the rare sequencing errors were corrected, it is predicted that the resulting nucleotide sequences would still be at least about 99.9% identical to the reference nucleotide sequences in SEQ ID NOs:1, 2, and 3. Thus, the present invention further provides nucleotide sequences that are at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1, 2, or 3 in a form which can

be readily used, analyzed and interpreted by the skilled artisan. Methods for determining whether a nucleotide sequence is at least 99.9% identical to a reference nucleotide sequence of the present invention are described below.

### *Nucleic Acid Molecules*

5           The present invention is directed to isolated nucleic acid fragments of the *M. jannaschii* genome. Such fragments include, but are not limited to, nucleic acid molecules encoding polypeptides (hereinafter open reading frames (ORFs)), nucleic acid molecules that modulate the expression of an operably linked ORF (hereinafter expression modulating fragments (EMFs)), nucleic acid molecules  
10           that mediate the uptake of a linked DNA fragment into a cell (hereinafter uptake modulating fragments (UMFs)), and nucleic acid molecules that can be used to diagnose the presence of *M. jannaschii* in a sample (hereinafter diagnostic fragments (DFs)).

15           By "isolated nucleic acid molecule(s)" is intended a nucleic acid molecule, DNA or RNA, that has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, purified (partially or substantially) DNA molecules in solution, and  
20           nucleic acid molecules produced synthetically. Isolated RNA molecules include *in vitro* RNA transcripts of the DNA molecules of the present invention.

25           In one embodiment, *M. jannaschii* DNA can be mechanically sheared to produce fragments about 15-20 kb in length, which can be used to generate a *M. jannaschii* DNA library by insertion into lambda clones as described in Example 1 below. Primers flanking an ORF described in Table 2(a) or 3 can then be generated using the nucleotide sequence information provided in SEQ ID NO:1, 2, or 3. The polymerase chain reaction (PCR) is then used to amplify and isolate the ORF from the lambda DNA library. PCR cloning is well known in the art. Thus, given SEQ ID NOs:1, 2, and 3, and Tables 2(a) and 3, it would be routine

to isolate any ORF or other representative fragment of the *M. jannaschii* genome. Isolated nucleic acid molecules of the present invention include, but are not limited to, single stranded and double stranded DNA, and single stranded RNA, and complements thereof.

5           Tables 2(a), 2(b) and 3 describe ORFs in the *M. jannaschii* genome. In particular, Table 2(a) (pages 67-115 below) indicates the location of ORFs (i.e., the position) within the *M. jannaschii* genome that putatively encode the recited protein based on homology matching with protein sequences from the organism appearing in parentheses (see the fourth column of Table 2(a)). The first  
10       column of Table 2(a) provides a name for each ORF. The second and third columns in Table 2(a) indicate an ORF's position in the nucleotide sequence provided in SEQ ID NO:1, 2 or 3. One of ordinary skill in the art will appreciate that the ORFs may be oriented in opposite directions in the *M. jannaschii* genome. This is reflected in columns 2 and 3. The fifth column of Table 2(a)  
15       indicates the percent identity of the protein sequence encoded by an ORF to the corresponding protein sequence from the organism appearing in parentheses in the fourth column. The sixth column of Table 2(a) indicates the percent similarity of the protein sequence encoded by an ORF to the corresponding protein sequence from the organism appearing in parentheses in the fourth  
20       column. The concepts of percent identity and percent similarity of two polypeptide sequences are well understood in the art and are described in more detail below. The eighth column in Table 2(a) indicates the length of the ORF in nucleotides. Each identified gene has been assigned a putative cellular role category adapted from Riley (Riley, M., *Microbiol. Rev.* 57:862 (1993)).

25           Table 2(b) (page 116 below) provides the single ORF identified by the present inventors that matches a previously published *M. jannaschii* gene. In particular, ORF MJ0479, which is 585 nucleotides in length and is positioned at nucleotides 1,050,508 to 1,049,948 in SEQ ID NO:1, shares 100% identity to the previously published *M. jannaschii* adenylate kinase gene.

30           Table 3 (pages 117-150 below) provides ORFs of the *M. jannaschii* genome that did not elicit a homology match with a known sequence from either



*M. jannaschii* or another organism. As above, the first column in Table 3 provides the ORF name and the second and third columns indicate an ORF's position in SEQ ID NO:1, 2, or 3.

Table 4 (page 151 below) provides genes of *M. jannaschii* that contain inteins.

In the above-described Tables, there are three groups of ORF names. The one thousand six hundred and eighty two ORFs named "MJ-" (MJ0001-MJ1682) were identified on the *M. jannaschii* chromosome (SEQ ID NO:1). The forty four ORFs named "MJECL-" (MJECL01-MJECL44) were identified on the large ECE (SEQ ID NO:2). The twelve ORFs named "MJECS-" (MJECS01-MJES12) were identified on the small ECE (SEQ ID NO:3).

Further details concerning the algorithms and criteria used for homology searches are provided in the Examples below. A skilled artisan can readily identify ORFs in the *Methanococcus jannaschii* genome other than those listed in Tables 2(a), 2(b) and 3, such as ORFs that are overlapping or encoded by the opposite strand of an identified ORF in addition to those ascertainable using the computer-based systems of the present invention.

Isolated nucleic acid molecules of the present invention include DNA molecules having a nucleotide sequence substantially different than the nucleotide sequence of an ORF described in Table 2(a) or 3, but which, due to the degeneracy of the genetic code, still encode a *M. jannaschii* protein. The genetic code is well known in the art. Thus, it would be routine to generate such degenerate variants.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of a *M. Jannaschii* protein encoded by an ORF described in Table 2(a) or 3. Non-naturally occurring variants may be produced using art-known mutagenesis techniques and include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or

non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the *M. jannaschii* protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

5 Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to (a) the nucleotide sequence of an ORF described in Table 2(a) or 3, (b) the  
10 nucleotide sequence of an ORF described in Table 2(a) or 3, but lacking the codon for the N-terminal methionine residue, if present, or (c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b). By a polynucleotide having a nucleotide sequence at least, for example, 95% identical to the reference *M. jannaschii* ORF nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence  
15 except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the ORF sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference ORF nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides  
20 up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within  
25 the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequence of a *M. jannaschii* ORF can be determined conventionally using known computer  
30 programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm

of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Preferred are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence of a *M. jannaschii* ORF that encode a functional polypeptide. By a "functional polypeptide" is intended a polypeptide exhibiting activity similar, but not necessarily identical, to an activity of the protein encoded by the *M. jannaschii* ORF. For example, the *M. jannaschii* ORF MJ1434 encodes an endonuclease that degrades DNA. Thus, a "functional polypeptide" encoded by a nucleic acid molecule having a nucleotide sequence, for example, 95% identical to the nucleotide sequence of MJ1434, will also degrade DNA. As the skilled artisan will appreciate, assays for determining whether a particular polypeptide is "functional" will depend on which ORF is used as the reference sequence. Depending on the reference ORF, the assay chosen for measuring polypeptide activity will be readily apparent in light of the role categories provided in Table 2(a).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of a reference ORF will encode a functional polypeptide. In fact, since degenerate variants all encode the same amino acid sequence, this will be clear to the skilled artisan even without performing a comparison assay for protein activity. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a functional polypeptide. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not

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likely to significantly affect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality. As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J.U. *et al.*, *supra*, and the references cited therein.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of a *M. jannaschii* ORF is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length that are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of a *M. jannaschii* ORF. By a fragment at least 20 nt in length, for example, is intended fragments that include 20 or more contiguous bases from the nucleotide sequence of a *M. jannaschii* ORF. Since *M. jannaschii* ORFs are listed in Tables 2(a) and 3 and the genome sequence has been provided, generating such DNA fragments would be routine to the skilled artisan. For

example, restriction endonuclease cleavage or shearing by sonication could easily be used to generate fragments of various sizes. Alternatively, such fragments could be generated synthetically.

5 Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of a *M. jannaschii* protein. Methods for determining such epitope-bearing portions are described in detail below.

10 In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide that hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, an ORF described in Table 2(a) or 3. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

15 By a polynucleotide that hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

20 Of course, polynucleotides hybridizing to a larger portion of the reference polynucleotide (e.g., a *M. jannaschii* ORF), for instance, a portion 50-500 nt in length, or even to the entire length of the reference polynucleotide, are also useful as probes according to the present invention, as are polynucleotides corresponding to most, if not all, of a *M. jannaschii* ORF.

By "expression modulating fragment" (EMF), is intended a series of nucleotides that modulate the expression of an operably linked ORF or EMF. A sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are fragments that induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event. EMF sequences can be identified within the *M. jannaschii* genome by their proximity to the ORFs described in Tables 2(a), 2(b), and 3. An intergenic segment, or a fragment of the intergenic segment, from about 10 to 200 nucleotides in length, taken 5' from any one of the ORFs of Tables 2(a), 2(b) or 3 will modulate the expression of an operably linked 3' ORF in a fashion similar to that found with the naturally linked ORF sequence. As used herein, an "intergenic segment" refers to the fragments of the *M. jannaschii* genome that are between two ORF(s) herein described. Alternatively, EMFs can be identified using known EMFs as a target sequence or target motif in the computer-based systems of the present invention.

The presence and activity of an EMF can be confirmed using an EMF trap vector. An EMF trap vector contains a cloning site 5' to a marker sequence. A marker sequence encodes an identifiable phenotype, such as antibiotic resistance or a complementing nutrition auxotrophic factor, which can be identified or assayed when the EMF trap vector is placed within an appropriate host under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence. A more detailed discussion of various marker sequences is provided below.

A sequence that is suspected as being an EMF is cloned in all three reading frames in one or more restriction sites upstream from the marker sequence in the EMF trap vector. The vector is then transformed into an appropriate host using known procedures and the phenotype of the transformed host is examined under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence.

By "uptake modulating fragment" (UMF), is intended a series of nucleotides that mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

By a "diagnostic fragment" (DF), is intended a series of nucleotides that selectively hybridize to *M. jannaschii* sequences. DFs can be readily identified by identifying unique sequences within the *M. jannaschii* genome, or by generating and testing probes or amplification primers consisting of the DF sequence in an appropriate diagnostic format for amplification or hybridization selectivity.

Each of the ORFs of the *M. jannaschii* genome disclosed in Tables 2(a) and 3, and the EMF found 5' to the ORF, can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes or diagnostic amplification primers to detect the presence *M. jannaschii* in a sample. This is especially the case with the fragments or ORFs of Table 3, which will be highly selective for *M. jannaschii*.

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)).

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Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

### ***Vectors and Host Cells***

The present invention further provides recombinant constructs comprising one or more fragments of the *M. jannaschii* genome. The recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which, for example, a *M. jannaschii* ORF is inserted. The vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs and UMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF or UMF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P<sub>R</sub>, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the



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appropriate vector and promoter is well within the level of ordinary skill in the art.

5 The present invention further provides host cells containing any one of the isolated fragments (preferably an ORF) of the *M. jannaschii* genome described herein. The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a procaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. *et al.*, *Basic*  
10 *Methods in Molecular Biology* (1986)). Host cells containing, for example, a *M. jannaschii* ORF can be used conventionally to produce the encoded protein.

### ***Polypeptides and Fragments***

15 The invention further provides an isolated polypeptide encoded by a *M. jannaschii* ORF described in Tables 2(a) or 3, or a peptide or polypeptide comprising a portion of the isolated polypeptide. The terms "peptide" and "oligopeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of at least two amino acids coupled by peptidyl linkages. The word "polypeptide" is used herein for chains containing more than ten amino acid residues.

20 It will be recognized in the art that some amino acid sequence of the *M. jannaschii* polypeptide can be varied without significant affect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. In general, it is possible to replace residues which form the  
25 tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein.

Thus, the invention further includes variations of a *M. jannaschii* protein encoded by an ORF described in Table 2(a) or 3 that show substantial protein

activity. Methods for assaying such "functional polypeptides" for protein activity are described above. Variations include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for strongly hydrophobic as a rule). Small changes or such "neutral" amino acid substitutions will generally have little effect on protein activity.

Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

As indicated in detail above, further guidance concerning amino acid changes that are likely to be phenotypically silent (i.e., are not likely to have a significant deleterious effect on function) can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990).

The fragment, derivative, variant or analog of a *M. jannaschii* polypeptide encoded by an ORF described in Table 2(a) or 3, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of a *M. jannaschii* ORF-encoded protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in a *M. jannaschii* ORF-encoded protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis

(Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule.

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of a *M. jannaschii* ORF-encoded protein can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

The polypeptides of the present invention include the proteins encoded by (a) an ORF described in Table 2(a) or 3 or (b) an ORF described in Table 2(a) or 3, but minus the codon for the N-terminal methionine residue, if present, as well as polypeptides that have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to a *M. jannaschii* ORF-encoded protein. Further polypeptides of the present invention include polypeptides at least 90% identical, more preferably at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to a *M. jannaschii* ORF-encoded protein.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (*Advances in Applied Mathematics* 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a *M. jannaschii* ORF-encoded protein is intended that the amino acid sequence of the polypeptide is

5 identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere  
10 between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide has an amino acid sequence at least 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of a *M. jannaschii* ORF-encoded protein can be determined  
15 conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine  
20 whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

25 As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting *M. jannaschii* protein expression.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The  
30 epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of

a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983). Antibodies that react with predetermined sites on proteins are described in *Science* 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer, peptides, especially those containing proline residues, usually are effective. Sutcliffe *et al.*, *supra*, at 661. For instance, 18 of 20 peptides designed according to these guidelines, containing 8-39 residues covering 75% of the sequence of the influenza virus hemagglutinin HA1 polypeptide chain, induced antibodies that reacted with the HA1 protein or intact virus; and 12/12 peptides from the MuLV polymerase and 18/18 from the rabies glycoprotein induced antibodies that precipitated the respective proteins.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. Thus, a high proportion of hybridomas obtained by fusion of spleen cells from donors immunized with an

antigen epitope-bearing peptide generally secrete antibody reactive with the native protein. Sutcliffe *et al.*, *supra*, at 663. The antibodies raised by antigenic epitope-bearing peptides or polypeptides are useful to detect the mimicked protein, and antibodies to different peptides may be used for tracking the fate of various regions of a protein precursor which undergoes post-translational processing. The peptides and anti-peptide antibodies may be used in a variety of qualitative or quantitative assays for the mimicked protein, for instance in competition assays since it has been shown that even short peptides (e.g., about 9 amino acids) can bind and displace the larger peptides in immunoprecipitation assays. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777. The anti-peptide antibodies of the invention also are useful for purification of the mimicked protein, for instance, by adsorption chromatography using methods well known in the art.

Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); and sequences containing proline residues are particularly preferred.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger

polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis. For instance, Houghten has described a simple method for synthesis of large numbers of peptides, such as 10-20 mg of 248 different 13 residue peptides representing single amino acid variants of a segment of the HA1 polypeptide which were prepared and characterized (by ELISA-type binding studies) in less than four weeks. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* 82:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986). In this procedure the individual resins for the solid-phase synthesis of various peptides are contained in separate solvent-permeable packets, enabling the optimal use of the many identical repetitive steps involved in solid-phase methods. A completely manual procedure allows 500-1000 or more syntheses to be conducted simultaneously. Houghten *et al.*, *supra*, at 5134.

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe *et al.*, *supra*; Wilson *et al.*, *supra*; Chow, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F. J. *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Generally, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine may be coupled to carrier using a linker such as m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 g peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for



instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. For instance, Geysen *et al.*, *supra*, discloses a procedure for rapid concurrent synthesis on solid supports of hundreds of peptides of sufficient purity to react in an enzyme-linked immunosorbent assay. Interaction of synthesized peptides with antibodies is then easily detected without removing them from the support. In this manner a peptide bearing an immunogenic epitope of a desired protein may be identified routinely by one of ordinary skill in the art. For instance, the immunologically important epitope in the coat protein of foot-and-mouth disease virus was located by Geysen *et al.* with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide analogs of the epitope-bearing peptides of the invention can be made routinely by this method. U.S. Patent No. 4,708,781 to Geysen (1987) further describes this method of identifying a peptide bearing an immunogenic epitope of a desired protein.

Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of

monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. *et al.* (1996) on Peralkylated Oligopeptide Mixtures discloses linear C<sub>1</sub>-C<sub>7</sub>-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

The entire disclosure of each document cited in this section on "Polypeptides and Peptides" is hereby incorporated herein by reference.

As one of skill in the art will appreciate, the polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been demonstrated, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*, *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric protein or protein fragment alone (Fountoulakis *et al.*, *J Biochem* 270:3958-3964 (1995)).

### ***Protein Function***

Each ORF described in Table 2(a) was assigned to biological role categories adapted from Riley, M., *Microbiology Reviews* 57(4):862 (1993)). This allows the skilled artisan to determine a function for each identified coding sequence. For example, a partial list of the *M. jannaschii* protein functions provided in Table 2(a) includes: methanogenesis, amino acid biosynthesis, cell

division, detoxification, protein secretion, transformation, central intermediary metabolism, energy metabolism, degradation of DNA, DNA replication, restriction, modification, recombination and repair, transcription, RNA processing, translation, degradation of proteins, peptides and glycopeptides, ribosomal proteins, translation factors, transport, tRNA modification, and drug and analog sensitivity. A more detailed description of several of these functions is provided in Example 1 below.

### ***Diagnostic Assays***

The present invention further provides methods to identify the expression of an ORF of the present invention, or homolog thereof, in a test sample, using one of the DFs or antibodies of the present invention. Such methods involve incubating a test sample with one or more of the antibodies or one or more of the DFs of the present invention and assaying for binding of the DFs or antibodies to components within the test sample.

Conditions for incubating a DF or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the DFs or antibodies of the present invention. Examples of such assays can be found in Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The test samples of the present invention include cells, protein or membrane extracts of cells. The test sample used in the above-described method

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will vary based on the assay format, nature of the detection method and the cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers including comprising: (a) a first container comprising one of the DFs or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound DF or antibody.

A compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or DF.

Types of detection reagents include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents that are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed DFs and antibodies of the present invention can be readily incorporated into one of the established kit formats that are well known in the art.

### *Screening Assay for Binding Agents*

Using the isolated proteins described herein, the present invention further provides methods of obtaining and identifying agents that bind to a protein encoded by a *M. jannaschii* ORF or to a fragment thereof.

5 The method involves:

- (a) contacting an agent with an isolated protein encoded by a *M. jannaschii* ORF, or an isolated fragment thereof; and
- (b) determining whether the agent binds to said protein or said fragment.

10 The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques. For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at  
15 random and are assayed for their ability to bind to the protein encoded by an ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one  
20 skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, Application of Synthetic Peptides: Antisense Peptides, In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY (1992), pp. 289-307,  
25 and Kaspczak *et al.*, *Biochemistry* 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly

screened or rationally designed and selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs that rely on the same EMF for expression control.

5 One class of DNA binding agents are those that contain nucleotide base residues that hybridize or form a triple helix by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives having base attachment capacity.

10 Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem.* 56:560 (1991);  
15 *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the  
20 sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

### ***Computer Related Embodiments***

25 The nucleotide sequence provided in SEQ ID NO:1, 2, or 3, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to the sequence provided in SEQ ID NO:1, 2, or 3, can be "provided" in a variety of mediums to facilitate use thereof. As used herein, provided refers to a manufacture, other than an isolated nucleic acid molecule, that contains a nucleotide sequence of the present invention, i.e., the nucleotide sequence provided in SEQ ID NO:1, 2, or 3, a representative fragment thereof, or a

nucleotide sequence at least 99.9% identical to SEQ ID NO:1, 2, or 3. Such a manufacture provides the *M. jannaschii* genome or a subset thereof (e.g., a *M. jannaschii* open reading frame (ORF)) in a form that allows a skilled artisan to examine the manufacture using means not directly applicable to examining the *M. jannaschii* genome or a subset thereof as it exists in nature or in purified form.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats

(e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide sequence of SEQ ID NO:1, 2, or 3, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1, 2, or 3, in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) and BLAZE (Brutlag *et al.*, *Comp. Chem.* 17:203-207 (1993)) search algorithms on a Sybase system can be used to identify open reading frames (ORFs) within the *M. jannaschii* genome that contain homology to ORFs or proteins from other organisms. Such ORFs are protein-encoding fragments within the *M. jannaschii* genome and are useful in producing commercially important proteins such as enzymes used in methanogenesis, amino acid biosynthesis, metabolism, fermentation, transcription, translation, RNA processing, nucleic acid and protein degradation, protein modification, and DNA replication, restriction, modification, recombination, and repair. A comprehensive list of ORFs encoding commercially important *M. jannaschii* proteins is provided in Tables 2(a) and 3.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the *M. jannaschii* genome. As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.



As indicated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory that can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention. As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the *M. jannaschii* genome that match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are available and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that during searches for commercially important fragments of the *M. jannaschii* genome, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is

5 formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymic active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

10 Thus, the present invention further provides an input means for receiving a target sequence, a data storage means for storing the target sequence and the homologous *M. jannaschii* sequence identified using a search means as described above, and an output means for outputting the identified homologous *M.*  
15 *jannaschii* sequence. A variety of structural formats for the input and output means can be used to input and output information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the *M. jannaschii* genome possessing varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or  
20 target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments of the  
25 *M. jannaschii* genome. For example, implementing software which implement the BLAST and BLAZE algorithms (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) can be used to identify open reading frames within the *M. jannaschii* genome. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the  
30 computer-based systems of the present invention.

One application of this embodiment is provided in Figure 4. Figure 4 provides a block diagram of a computer system 102 that can be used to implement the present invention. The computer system 102 includes a processor 106 connected to a bus 104. Also connected to the bus 104 are a main memory 108 (preferably implemented as random access memory, RAM) and a variety of  
35 secondary storage devices 110, such as a hard drive 112 and a removable medium

storage device 114. The removable medium storage device 114 may represent, for example, a floppy disk drive, a CD-ROM drive, a magnetic tape drive, etc. A removable storage medium 116 (such as a floppy disk, a compact disk, a magnetic tape, etc.) containing control logic and/or data recorded therein may be inserted into the removable medium storage device 114. The computer system 102 includes appropriate software for reading the control logic and/or the data from the removable medium storage device 114 once inserted in the removable medium storage device 114.

A nucleotide sequence of the present invention may be stored in a well known manner in the main memory 108, any of the secondary storage devices 110, and/or a removable storage medium 116. Software for accessing and processing the genomic sequence (such as search tools, comparing tools, etc.) reside in main memory 108 during execution.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

### *Experimental*

#### *Complete genome sequence of the methanogenic archaeon, Methanococcus jannaschii*

##### *Example 1*

A whole genome random sequencing method (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)) was used to obtain the complete genome sequence for *M. jannaschii*. A small insert plasmid library (2.5 Kbp average insert size) and a large insert lambda library (16 Kbp average insert size) were used as substrates for sequencing. The lambda library was used to form a genome scaffold and to verify the orientation and integrity of the contigs formed from the assembly of sequences from the plasmid library. All clones were sequenced from both ends to aid in ordering of contigs during the sequence assembly process. The average length of sequencing reads was 481 bp. A total of 36,718 sequences were assembled by means of the TIGR

Assembler (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995); Sutton G., *et al.*, *Genome Sci. Tech.* 1:9 (1995)). Sequence and physical gaps were closed using a combination of strategies (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)). The colinearity of the *in vivo* genome to the genome sequence was confirmed by comparing restriction fragments from six, rare cutter, restriction enzymes (Aat II, BamHI, Bgl II, Kpn I, Sma I, and Sst II) to those predicted from the sequence data. Additional confidence in the colinearity was provided by the genome scaffold produced by sequence pairs from 339 large-insert lambda clones, which covered 88% of the main chromosome. Open reading frames (ORFs) and predicted protein-coding regions were identified as described (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)) with some modification. In particular, the statistical prediction of *M. jannaschii* genes was performed with GeneMark (Borodovsky, M. & McIninch, J. *Comput. Chem.* 17:123 (1993)). Regular GeneMark uses nonhomogeneous Markov models derived from a training set of coding sequences and ordinary Markov models derived from a training set of noncoding sequences. Only a single 16S ribosomal RNA sequence of *M. jannaschii* was available in the public sequence databases before the whole genome sequence described here. Thus, the initial training set to determine parameters of a coding sequence Markov model was chosen as a set of ORFs >1000 nucleotides (nt). As an initial model for non-coding sequences, a zero-order Markov model with genome-specific nucleotide frequencies was used. The initial models were used at the first prediction step. The results of the first prediction were then used to compile a set of putative genes used at the second training step. Alternate rounds of training and predicting were continued until the set of predicted genes stabilized and the parameters of the final fourth-order model of coding sequences were derived. The regions predicted as noncoding were then used as a training set for a final model for noncoding regions. Cross-validation simulations demonstrated that the GeneMark program trained as described above was able to correctly identify coding regions of at least 96 nt in 94% of the cases and noncoding regions of the

same length in 96% of the cases. These values assume that the self-training method produced correct sequence annotation for compiled control sets. Comparison with the results obtained by searches against a nonredundant protein database (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)) demonstrated that almost all genes identified by sequence similarity were predicted by the GeneMark program as well. This observation provides additional confidence in genes predicted by GeneMark whose protein translations did not show significant similarity to known protein sequences. The predicted protein-coding regions were search against the Blocks database (Henikoff, S. & Henikoff, J.G., *Genomics* 19:97 (1994)) by means of BLIMPS (Wallace, J.C. & Henikoff, S., *CABIOS* 8:249 (1992)) to verify putative identifications and to identify potential functional motifs in predicted protein-coding regions that had no database match. Genes were assigned to known metabolic pathways. When a gene appeared to be missing from a pathway, the unassigned ORFs and the complete *M. jannaschii* genome sequence were searched with specific query sequences or motifs from the Blocks database. Hydrophobicity plots were performed on all predicted protein-coding regions by means of the Kyte-Doolittle algorithm (Kyte, J. & Doolittle, R.F., *J. Mol. Biol.* 157:105 (1982)) to identify potentially functionally relevant signatures in these sequences.

The *M. jannaschii* genome comprises three physically distinct elements: i) a large circular chromosome of 1,664,976 base pairs (bp) (SEQ ID NO:1), which contains 1682 predicted protein-coding regions and has a G+C content of 31.4%; ii) a large circular extrachromosomal element (ECE) (Zhao, H., *et al.*, *Arch. Microbiol.* 150:178 (1988)) of 58,407 bp (SEQ ID NO:2), which contains 44 predicted protein coding regions and has a G+C content of 28.2%; and iii) a small circular ECE (Zhao, H., *et al.*, *Arch. Microbiol.* 150:178 (1988)) of 16,550 bp (SEQ ID NO:3), which contains 12 predicted protein coding regions, and has a G+C content of 28.8%. With respect to its shape, size, G+C content, and gene density the main chromosome resembles that of *H. influenzae*. However, here the resemblance stops.

Of the 1743 predicted protein-coding regions reported previously for *H. influenzae*, 78% had a match in the public sequence database (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)). Of these, 58% were matches to genes with reasonably well defined function, while 20% were matches to genes whose function was undefined. Similar observations were made for the *M. genitalium* genome (Fleischmann, R.D., *et al.*, *Science* 269:496 (1995); Fraser, C.M., *et al.*, *Science* 270:397 (1995)). Eighty-three percent of the predicted protein coding regions from *M. genitalium* have a counterpart in the *H. influenzae* genome. In contrast, only 38% of the predicted protein-coding regions from *M. jannaschii* match a gene in the database that could be assigned a putative cellular role with high confidence; 6% of the predicted protein-coding regions had matches to hypothetical proteins (Tables 2-3). Approximately 100 genes in *M. jannaschii* had marginal similarity to genes or segments of genes from the public sequence databases and could not be assigned a putative cellular role with high confidence. Only 11% of the predicted protein-coding regions from *H. influenzae* and 17% of the predicted protein coding regions from *M. genitalium* matched a predicted protein coding region from *M. jannaschii*. Clearly the *M. jannaschii* genome, and undoubtedly, therefore, all archaeal genomes are remarkably unique, as the phylogenetic position of these organisms would suggest.

Energy production in *M. jannaschii* occurs via the reduction of CO<sub>2</sub> with H<sub>2</sub> to produce methane. Genes for all of the known enzymes and enzyme complexes associated with methanogenesis (DiMarco, A.A., *et al.*, *Ann. Rev. Biochem.* 59:355 (1990)) were identified in *M. jannaschii*, the sequence and order of which are typical of methanogens. *M. jannaschii* appears to use both H<sub>2</sub> and formate as substrates for methanogenesis, but lacks the genes to use methanol or acetate. The ability to fix nitrogen has been demonstrated in a number of methanogens (Belay, N., *et al.*, *Nature* 312:286 (1984)) and all of the genes necessary for this pathway have been identified in *M. jannaschii* (Tables 2-3). In addition to its anabolic pathways, several scavenging molecules have been

identified in *M. jannaschii* that probably play a role in importing small organic compounds, such as amino acids, from the environment (Tables 2-3).

Three different pathways are known for the fixation of CO<sub>2</sub> into organic carbon: the non-cyclic, reductive acetyl-coenzyme A-carbon monoxide dehydrogenase pathway (Ljungdahl-Wood pathway), the reductive trichloroacetic acid (TCA) cycle, and the Calvin cycle. Methanogens fix carbon by the Ljungdahl-Wood pathway (Wood, H.G., *et al.*, *TIBS* 11:14 (1986)), which is facilitated by the carbon monoxide dehydrogenase enzyme complex (CODH) (Blaat, M., *Antonie van Leeuwenhoek* 66:187 (1994)). The complete Ljungdahl-Wood pathway, encoded in the *M. jannaschii* genome, depends on the methyl carbon in methanogenesis; however, methanogenesis can occur independently of carbon fixation.

Although genes encoding two enzymes required for gluconeogenesis (glucopyruvate oxidoreductase and phosphoenolpyruvate synthase) were found in the *M. jannaschii* genome, genes encoding other key intermediates of gluconeogenesis (fructose biphosphatase and fructose 1,6-bisphosphate aldolase) were not been identified. Glucose catabolism by glycolysis also requires the aldolase, as well as phosphofructokinase, an enzyme that also was not found in *M. jannaschii* and has not been detected in any of the Archaea. In addition, genes specific for the Entner-Doudoroff pathway, an alternative pathway used by some microbes for the catabolism of glucose, were not identified in the genomic sequence. The presence of a number of nearly complete metabolic pathways suggests that some key genes are not recognizable at the sequence level, although we cannot exclude the possibility that *M. jannaschii* may use alternative metabolic pathways.

In general, *M. jannaschii* genes that encode proteins involved in the transport of small inorganic ions into the cell are homologs of bacterial genes. The genome includes many representatives of the ABC transporter family, as well as genes for exporting heavy metals (e.g., the chromate-resistance protein) and other toxic compounds (e.g., the *norA* drug efflux pump locus).

More than 20 predicted protein-coding regions have sequence similarity to polysaccharide biosynthetic enzymes. These genes have only bacterial homologs or are most closely related to their bacterial counterparts. The identified polysaccharide biosynthetic genes in *M. jannaschii* include those for the interconversion of sugars, activation of sugars to nucleotide sugars, and glycosyltransferases for the polymerization of nucleotide sugars into oligo- and polysaccharides that are subsequently incorporated into surface structures (Hartmann, E. and König, H., *Arch. Microbiol.* 151:274 (1989)). In an arrangement reminiscent of bacterial polysaccharide biosynthesis genes, many of the genes for *M. jannaschii* polysaccharide production are clustered together (Tables 2-3). The G+C content in this region is <95% of that in the rest of the *M. jannaschii* genome. A similar observation was made in *Salmonella typhimurium* (Jiang, X.M., et al., *Mol. Microbiol.* 5:695 (1991)) in which the gene cluster for lipopolysaccharide O antigen has a significantly lower G+C ratio than the rest of the genome. In that case, the difference in G+C content was interpreted as meaning that the region originated by lateral transfer from another organism.

Of the three main multicomponent information processing systems (transcription, translation, and replication), translation appears the most universal in its overall makeup in that the basic translation machinery is similar in all three domains of life. *M. jannaschii* has two ribosomal RNA operons, designated A and B, and a separate 5S RNA gene that is associated with several transfer RNAs (tRNAs). Operon A has the organization, 16S - 23S - 5S, whereas operon B lacks the 5S component. An alanine tRNA is situated in the spacer region between the 16S and 23S subunits in both operons. The majority of proteins associated with the ribosomal subunits (especially the small subunit) are present in both Bacteria and Eukaryotes. However, the relatively protein-rich eukaryotic ribosome contains additional ribosomal proteins not found in the bacterial ribosome. A smaller number of bacteria-specific ribosomal proteins exist as well. The *M. jannaschii* genome contains all ribosomal proteins that are common to eukaryotes and bacteria. It shows no homologs of the bacterial-specific ribosomal proteins, but does possess homologs of a number of the eukaryotic-specific ones.



Homologs of all archaea-specific ribosomal proteins that have been reported to date (Lechner, K., *et al.*, *J. Mol. Evol.* 29:20 (1989); Köpke, A.K.E. and Wittmann-Liebold, B., *Can. J. Microbiol.* 35:11 (1989)) are found in *M. jannaschii*.

As previously shown for other archaea (Iwabe, N., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9355 (1989); Gogarten J.P., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:6661 (1989); Brown, J.R. and Doolittle, W.F., *Proc. Natl. Acad. Sci. USA* 92:2441 (1995)), the *Methanococcus* translation elongation factors EF-1 $\alpha$  (EF-Tu in bacteria) and EF-2 (EF-G in bacteria) are most similar to their eukaryotic counterparts. In addition, the *M. jannaschii* genome contains 11 translation initiation factor genes. Three of these genes encode the subunits homologous to those of the eukaryotic IF-2, and are reported here in the Archaea for the first time. A fourth initiation factor gene that encodes a second IF-2 is also found in *M. jannaschii*. This additional IF-2 gene is most closely related to the yeast protein FUN12 which, in turn, appears to be a homolog of the bacterial IF-2. It is not known which of the two IF-2-like initiation factors identified in *M. jannaschii* plays a role in directing the initiator tRNA to the start site of the mRNA. The fifth identified initiation factor gene in *M. jannaschii* encodes IF-1A, which has no bacterial homolog. The sixth gene encodes the hypusine-containing initiation factor eIF-5a. Two subunits of the translation initiation factor eIF-2B were identified in *M. jannaschii*. Finally, three putative adenosine 5'-triphosphate (ATP)-dependent helicases were identified that belong to the eIF-4a family of translation initiation factors.

Thirty-seven tRNA genes were identified in the *M. jannaschii* genome. Almost all amino acids encoded by two codons have a single tRNA, except for glutamic acid, which has two. Both an initiator and an internal methionyl tRNA are present. The two pyrimidine-ending isoleucine codons are covered by a single tRNA, while the third (AUA) seems covered by a related tRNA having a CAU anticodon. A single tRNA appears to cover the three isoleucine codons. Those amino acids encoded by four codons each have two tRNAs, one to cover the Y-, the other the R-ending, codons. Valine has a third tRNA, which is

specific for the GUG codon; and alanine has three tRNAs (two of which are in the spacer regions separating the 16S and 23S subunits in the two ribosomal RNA operons). Leucine, serine and arginine, all of which have six codons, each possess three corresponding tRNAs. The genes for the internal methionine and tryptophan tRNAs contain introns in the region of their anti-codon loops.

A tRNA also exists for selenocysteine (UGA codon). At least four genes in *M. jannaschii* contain internal stop codons that are potential selenocysteine codons: the  $\alpha$  chain of formate dehydrogenase, coenzyme F420 reducing hydrogenase,  $\beta$ -chain tungsten formyl methanofuran dehydrogenase, and a heterodisulfide reductase. Three genes with a putative role in selenocysteine metabolism were identified by their similarity to the *sel* genes from other organisms (Tables 2-3).

Recognizable homologs for four of the aminoacyl-tRNA synthetases (glutamine, asparagine, lysine, and cysteine) were not identified in the *M. jannaschii* genome. The absence of a glutaminyl-tRNA synthetase is not surprising in that a number of organisms, including at least one archaeon, have none (Wilcox, M., *Eur. J. Biochem.* 11:405 (1969); Martin, N.C., *et al.*, *J. Mol. Biol.* 101:285 (1976); Martin, N.C., *et al.*, *Biochemistry* 16:4672 (1977); Schon, A., *et al.*, *Biochimie* 70:391 (1988); Soll, D. and RajBhandary, U., Eds. *Am. Soc. for Microbiol.* (1995)). In these instances, glutaminyl tRNA charging involves a post-charging conversion mechanism whereby the tRNA is charged by the glutamyl-tRNA synthetase with glutamic acid, which then is enzymatically converted to glutamine. A post-charging conversion is also involved in selenocysteine charging via the seryl-tRNA synthetase. A similar mechanism has been proposed for asparagine charging, but has never been demonstrated (Wilcox, M., *Eur. J. Biochem.* 11:405 (1969); Martin, N.C., *et al.*, *J. Mol. Biol.* 101:285 (1976); Martin, N.C., *et al.*, *Biochemistry* 16:4672 (1977); Schon, A., *et al.*, *Biochimie* 70:391 (1988); Soll, D. and RajBhandary, U., Eds. *Am. Soc. for Microbiol.* (1995)). The inability to find homologs of the lysine and cysteine aminoacyl-tRNA synthetases is surprising because bacterial and eukaryotic versions in each instance show clear homology.

Aminoacyl-tRNA synthetases of *M. jannaschii* and other archaea resemble eukaryotic synthetases more closely than they resemble bacterial forms. The tryptophanyl synthetase is one of the more notable examples, because the *M. jannaschii* and eukaryotic version do not appear to be specifically related to the bacterial version (de Pouplana, R., *et al.*, *Proc. Natl. Acad. Sci., USA* 93:166 (1996)). Two versions of the glycyl synthetase are known in bacteria, one that is very unlike the version found in Archaea and Eukaryote and one that is an obvious homolog of it (Wagner, E.A., *et al.*, *J. Bacteriol.* 177:5179 (1995); Logan, D.T., *et al.*, *EMBO J.* 14:4156 (1995)).

Eleven genes encoding subunits of the DNA-dependent RNA polymerase were identified in the *M. jannaschii* genome. The sequence similarity between the subunits and their homologs in *Sulfolobus acidocaldarius* supports the evolutionary unity of the archaeal polymerase complex (Woese, C.R. and Wolfe, R.S., Eds. *The Bacteria*, vol. VIII (Academic Press, NY, 1985); Langer, D., *et al.*, *Proc. Natl. Acad. Sci.* 92:5768 (1995); Lanzendoerfer, M. *et al.*, *System. Appl. Microbiol.* 16:656 (1994)). All of the subunits found in *M. jannaschii* show greater similarity to their eukaryotic counterparts than to the bacterial homologs. The genes encoding the five largest subunits (A', A'', B', B'', D) have homologs in all organisms. Six genes encode subunits shared only by Archaea and Eukaryotes (E, H, K, L, and N). The *M. jannaschii* homolog of the *S. acidocaldarius* subunit E is split into two genes designated E' and E''. *Sulfolobus acidocaldarius* also contains two additional small subunits of RNA polymerase, designated G and F, that have no counterparts in either Bacteria or Eukaryotes. No homolog of these subunits was identified in *M. jannaschii*.

The archaeal transcription initiation system is essentially the same as that found in Eukaryotes, and is radically different from the bacterial version (Klenk, H.P. and Doolittle, W.F., *Curr. Biol.* 4:920 (1994)). The central molecules in the former systems are the TATA-binding protein (TBP) and transcription factor B (TFIIB and TFIIB in Eukaryotes, or simply TFB). In the eukaryotic systems, TBP and TFB are parts of larger complexes, and additional factors (such as

TFIIA and TFIIF) are used in the transcription process. However, the *M. jannaschii* genome does not contain obvious homologs of TFIIA and TFIIF.

Several components of the replication machinery were identified in *M. jannaschii*. The *M. jannaschii* genome appears to encode a single DNA-dependent polymerase that is a member of the B family of polymerases (Bernard, A., *et al.*, *EMBO J.* 6:4219 (1987); Cullman, G., *et al.*, *Molec. Cell Biol.* 15:4661 (1995); Uemori, T., *et al.*, *J. Bacteriol.* 117:2164 (1995); Delarue, M., *et al.*, *Prot. Engineer.* 3:461 (1990); Gavin, K.A., *et al.*, *Science* 270:1667 (1995)). The polymerase shares sequence similarity and three motifs with other family B polymerases, including eukaryotic  $\alpha$ ,  $\gamma$ , and  $\epsilon$  polymerases, bacterial polymerase II, and several archaeal polymerases. However, it is not homologous to bacterial polymerase I and has no homologs in *H. influenzae* or *M. genitalium*.

Primer recognition by the polymerase takes place through a structure-specific DNA binding complex, the replication factor complex (rfc) (Bernard, A., *et al.*, *EMBO J.* 6:4219 (1987); Cullman, G., *et al.*, *Molec. Cell Biol.* 15:4661 (1995); Uemori, T., *et al.*, *J. Bacteriol.* 117:2164 (1995); Delarue, M., *et al.*, *Prot. Engineer.* 3:461 (1990); Gavin, K.A., *et al.*, *Science* 270:1667 (1995)). In humans and yeast, the rfc is composed of five proteins: a large subunit and four small subunits that have an associated adenosine triphosphatase (ATPase) activity stimulated by proliferating cell nuclear antigen (PCNA). Two genes in *M. jannaschii* are putative members of a eukaryotic-like replication factor complex. One of the genes in *M. jannaschii* is a putative homolog of the large subunit of the rfc, whereas the second is a putative homolog of one of the small subunits. Among Eukaryotes, the rfc proteins share sequence similarity in eight signature domains (Bernard, A., *et al.*, *EMBO J.* 6:4219 (1987); Cullman, G., *et al.*, *Molec. Cell Biol.* 15:4661 (1995); Uemori, T., *et al.*, *J. Bacteriol.* 117:2164 (1995); Delarue, M., *et al.*, *Prot. Engineer.* 3:461 (1990); Gavin, K.A., *et al.*, *Science* 270:1667 (1995)). Domain I is conserved only in the large subunit among Eukaryotes and is similar in sequence to DNA ligases. This domain is missing in the large-subunit homolog in *M. jannaschii*. The remaining domains in the two *M. jannaschii* genes are well-conserved relative to the eukaryotic homologs. Two

features of the sequence similarity in these domains are of particular interest. First, domain II (an ATPase domain) of the small-subunit homolog is split between two highly conserved amino acids (lysine and threonine) by an intervening sequence of unknown function. Second, the sequence of domain VI has regions that are useful for distinguishing between bacterial and eukaryotic rfc proteins (Bernard, A., *et al.*, *EMBO J.* 6:4219 (1987); Cullman, G., *et al.*, *Molec. Cell Biol.* 15:4661 (1995); Uemori, T., *et al.*, *J. Bacteriol.* 117:2164 (1995); Delarue, M., *et al.*, *Prot. Engineer.* 3:461 (1990); Gavin, K.A., *et al.*, *Science* 270:1667 (1995)); the rfc sequence for *M. jannaschii* shares the characteristic eukaryotic signature in this domain.

We have attempted to identify an origin of replication by searching the *M. jannaschii* genome sequence with a variety of bacterial and eukaryotic replication-origin consensus sequences. Searches with oriC, ColE1, and autonomously replicating sequences from yeast (Bernard, A., *et al.*, *EMBO J.* 6:4219 (1987); Cullman, G., *et al.*, *Molec. Cell Biol.* 15:4661 (1995); Uemori, T., *et al.*, *J. Bacteriol.* 117:2164 (1995); Delarue, M., *et al.*, *Prot. Engineer.* 3:461 (1990); Gavin, K.A., *et al.*, *Science* 270:1667 (1995)) did not identify an origin of replication. With respect to the related cellular processes of replication initiation and cell division, the *M. jannaschii* genome contains two genes that are putative homologs of Cdc54, a yeast protein that belongs to a family of putative DNA replication initiation proteins (Whitbred, L.A. and Dalton, S., *Gene* 155:113 (1995)). A third potential regulator of cell division in *M. jannaschii* is 55% similar at the amino acid level to *pelota*, a *Drosophila* protein involved in the regulation of the early phases of meiotic and mitotic cell division (Eberhart, C.G. and Wasserman, S.A., *Development* 121:3477 (1995)).

In contrast to the putative rfc complex and the initiation of DNA replication, the cell division proteins from *M. jannaschii* most resemble their bacterial counterparts (Rothfield, L.I. and Zhao, C.R., *Cell* 84:183 (1996); Lutkenhaus, J., *Curr. Opp. Gen. Devel.* 3:783 (1993)). Two genes similar to that encoding FtsZ, a ubiquitous bacterial protein, are found in *M. jannaschii*. FtsZ

is a polymer-forming, guanosine triphosphate (GTP)-hydrolyzing protein with tubulin-like elements; it is localized to the site of septation and forms a constricting ring between the dividing cells. One gene similar to FtsJ, a bacterial cell division protein of undetermined function, also is found in *M. jannaschii*.  
5 Three additional genes (MinC, MinD, and MinE) function in concert in Bacteria to determine the site of septation during cell division. In *M. jannaschii*, three MinD-like genes were identified, but none for MinC or MinE. Neither spindle-associated proteins characteristic of eukaryotic cell division nor bacterial  
10 mechanochemical enzymes necessary for partitioning the condensed chromosomes were detected in the *M. jannaschii* genome. Taken together, these observations raise the possibility that cell division in *M. jannaschii* might occur via a mechanism specific for the Archaea.

The structural and functional conservation of the signal peptide of secreted proteins in Archaea, Bacteria, and Eukaryotes suggests that the basic  
15 mechanisms of membrane targeting and translocation may be similar among all three domains of life. The secretory machinery of *M. jannaschii* appears a rudimentary apparatus relative to that of bacterial and eukaryotic systems and consists of (i) a signal peptidase (SP) that cleaves the signal peptide of  
20 translocating proteins, (ii) a preprotein translocase that is the major constituent of the membrane-localized translocation channel, (iii) a ribonucleoprotein complex (signal recognition particle, SRP) that binds to the signal peptide and guides nascent proteins to the cell membrane, and (iv) a docking protein that acts as a receptor for the SRP. The 7S RNA component of the SRP from *M. jannaschii* shows a highly conserved structural domain shared by other Archaea,  
25 Bacteria, and Eukaryotes (Kaine, B.P. and Merkel, V.L., *J. Bacteriol.* 171:4261 (1989); Poritz, M.A. *et al.*, *Cell* 55:4 (1988)). However, the predicted secondary structure of the 7S RNA SRP component in Archaea is more like that found in Eukaryotes than in Bacteria (Kaine, B.P. and Merkel, V.L., *J. Bacteriol.* 171:4261 (1989); Poritz, M.A. *et al.*, *Cell* 55:4 (1988)). The SP and docking proteins from  
30 *M. jannaschii* are most similar to their eukaryotic counterparts; the translocase is most similar to the SecY translocation-associated protein in *Escherichia coli*.

A second distinct signal peptide is found in the flagellin genes of *M. jannaschii*. Alignment of flagellin genes from *M. voltae* (Faguy, D.M., *et al.*, *Can. J. Microbiol.* 40:67 (1994); Kalmokoff, M.L., *et al.*, *Arch. Microbiol.* 157:481 (1992)) and *M. jannaschii* reveals a highly conserved NH<sub>2</sub>-terminus (31 of the first 50 residues are identical in all of the mature flagellins). The peptide sequence of the *M. jannaschii* flagellin indicates that the protein is cleaved after the canonical Gly-12 position, and it is proposed to be similar to type-IV pilins of Bacteria (Faguy, D.M., *et al.*, *Can. J. Microbiol.* 40:67 (1994); Kalmokoff, M.L., *et al.*, *Arch. Microbiol.* 157:481 (1992)).

Five histone genes are present in the *M. jannaschii* genome--three on the main chromosome and two on the large ECE. These genes are homologs of eukaryotic histones (H2a, H2b, H3, and H4) and of the eukaryotic transcription-related CAAT-binding factor CBF-A (Sandman, K., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:5788 (1990)). The similarity between archaeal and eukaryotic histones suggests that the two groups of organisms resemble one another in the roles histones play both in genome supercoiling dynamics and in gene expression. The five *M. jannaschii* histone genes show greatest similarity among themselves even though a histone sequence is available from the closely related species, *Methanococcus voltae*. This intraspecific similarity suggests that the gene duplications that produced the five histone genes occurred on the *M. jannaschii* lineage per se.

Self-splicing portions of a peptide sequence that generally encode a DNA endonuclease activity are called inteins, in analogy to introns (Kane, P.M., *et al.*, *Science* 250:651 (1990); Hirata, R., *et al.*, *J. Biol. Chem.* 265:6726 (1990); Cooper, A. and Stevens, T., *TIBS* 20:351 (1995); Xu, M.Q., *et al.*, *Cell* 75:1371 (1993); Perler *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5577 (1992); Cooper *et al.*, *EMBO J.* 12:2575 (1993); Michel *et al.*, *Biochimie* 64:867 (1992); Pietrovski S., *Prot. Sci.* 3:2340 (1994). Most inteins in the *M. jannaschii* genome were identified by (i) similarity of the bounding exteins to other proteins, (ii) similarity of the inteins to those previously described, (iii) presence of the dodecapeptide endonuclease motifs, and (iv) canonical intein-extein junction sequences. In two

instances (MJ0832 and MJ0043), the similarity to other database sequences did not unambiguously define the NH<sub>2</sub>-terminal extein-intein junction, so it was necessary to rely on consensus sequences to select the putative site. The inteins in MJ1042 and MJ0542 have previously uncharacterized COOH-terminal splice junctions, GNC and FNC, respectively).

The sequences remaining after an intein is excised are called exteins, in analogy to exons. Exteins are spliced together after the excision of one or more inteins to form functional proteins. The biological significance and role of inteins are not clearly understood (Kane, P.M., *et al.*, *Science* 250:651 (1990); Hirata, R., *et al.*, *J. Biol. Chem.* 265:6726 (1990); Cooper, A. and Stevens, T., *TIBS* 20:351 (1995); Xu, M.Q., *et al.*, *Cell* 75:1371 (1993); Perler *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5577 (1992); Cooper *et al.*, *EMBO J.* 12:2575 (1993); Michel *et al.*, *Biochimie* 64:867 (1992); Pietrokovski S., *Prot. Sci.* 3:2340 (1994)). Fourteen genes in the *M. jannaschii* genome contain 18 putative inteins, a significant increase in the approximately 10 intein-containing genes that have been described (Kane, P.M., *et al.*, *Science* 250:651 (1990); Hirata, R., *et al.*, *J. Biol. Chem.* 265:6726 (1990); Cooper, A. and Stevens, T., *TIBS* 20:351 (1995); Xu, M.Q., *et al.*, *Cell* 75:1371 (1993); Perler *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5577 (1992); Cooper *et al.*, *EMBO J.* 12:2575 (1993); Michel *et al.*, *Biochimie* 64:867 (1992); Pietrokovski S., *Prot. Sci.* 3:2340 (1994)) (Table 4). The only previously described inteins in the Archaea are in the DNA polymerase genes of the Thermococcales (Kane, P.M., *et al.*, *Science* 250:651 (1990); Hirata, R., *et al.*, *J. Biol. Chem.* 265:6726 (1990); Cooper, A. and Stevens, T., *TIBS* 20:351 (1995); Xu, M.Q., *et al.*, *Cell* 75:1371 (1993); Perler *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5577 (1992); Cooper *et al.*, *EMBO J.* 12:2575 (1993); Michel *et al.*, *Biochimie* 64:867 (1992); Pietrokovski S., *Prot. Sci.* 3:2340 (1994)). The *M. jannaschii* DNA polymerase gene has two inteins in the same locations as those in *Pyrococcus* sp. strain KOD1. In this case, the exteins exhibit 46% amino acid identity, whereas intein 2 of the two organisms has only 33% identity. This divergence suggests that intein 2 has not been recently (laterally) transferred between the Thermococcales and *M. jannaschii*. In contrast, the intein 1



sequences are 56% identical, more than that of the gene containing them, and comparable to the divergence of inteins within the Thermococcales. This high degree of sequence similarity might be the result of an intein transfer more recent than the splitting of these species. The large number of inteins found in *M. jannaschii* led us to question whether these inteins have been increasing in number by moving within the genome. If this were so, we would expect to find some pairs of inteins that are particularly similar. Comparisons of these and other available intein sequences showed that the closest relationships are those noted above linking the DNA polymerase inteins to correspondingly positioned elements in the Thermococcales. Within *M. jannaschii*, the highest identity observed was 33% for a 380-bp portion of two inteins. This finding suggests that the diversification of the inteins predates the divergence of the *M. jannaschii* and *Pyrococcus* DNA polymerases.

Three families of repeated genetic elements were identified in the *M. jannaschii* genome. Within two of the families, at least two members were identified as ORFs with a limited degree of sequence similarity to bacterial transposases. Members of the first family, designated *ISAMJI*, are repeated 10 times on the main chromosome and once on the large ECE (Fig. 2). There is no sequence similarity between the IS elements in *M. jannaschii* and the *ISM1* mobile element described previously for *Methanobrevibacter smithii* (Hamilton, P.T. et al., *Mol. Gen. Genet.* 200:47 (1985)). Two members of this family were identified as ORFs and are 27% identical (at the amino acid sequence level) to a transposase from *Bacillus thuringiensis* (IS240; GenBank accession number M23741). Relative to these two members, the remaining members of the *ISAMJI* family are missing an internal region of several hundred nucleotides (Fig. 2). With one exception, all members of this family end with 16-bp terminal inverted repeats typical of insertion sequences. One member is missing the terminal repeat at its 5' end. The second family consists of two ORFs that are identical across 928 bp. The ORFs are 23% identical at the amino acid sequence level to the COOH-terminus of a transposase from *Lactococcus lactis* (IS982; GenBank

accession number L34754). Neither of the members of the second family contains terminal inverted repeats.

5 Eighteen copies of the third family of repeated genetic structures (Fig. 3) are distributed fairly evenly around the *M. jannaschii* genome. Unlike the genetic elements described above, none of the components of this repeat unit appears to have coding potential. The repeat structure is composed of a long segment followed by one to 25 tandem repetitions of a short segment. The short segments are separated by sequence that is unique within and among the complete repeat structure. Three similar types of short segments were identified; however, the type of short repeat is consistent within each repeat structure, except for variation of the last short segment in six repeat structures. Similar tandem repeats of short segments have been observed in Bacteria and other Archaea (Mojica, F.J.M., *et al.*, *Mol. Micro.* 17:85 (1995)) and have been hypothesized to participate in chromosome partitioning during cell division.

15 The 16-kbp ECE from *M. jannaschii* contains 12 ORFs, none of which had a significant full-length match to any published sequence. The 58-kbp ECE contains 44 predicted protein-coding regions, 5 of which had matches to genes in the database. Two of the genes are putative archaeal histones, one is a sporulation-related protein (SOJ protein), and two are type I restriction modification enzymes. There are several instances in which predicted protein-coding regions or repeated genetic elements on the large ECE have similar counterparts on the main chromosome of *M. jannaschii*. The degree of nucleotide sequence similarity between genes present on both the ECE and the main chromosome ranges from 70 to 90%, suggesting that there has been relatively recent exchange of at least some genetic material between the large ECE and the main chromosome.

25 All the predicted protein-coding regions from *M. jannaschii* were searched against each other in order to identify families of paralogous genes (genes related by gene duplication, not speciation). The initial criterion for grouping paralogs was >30% amino acid sequence identity over 50 consecutive amino acid residues. Groups of predicted protein-coding regions were then

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aligned and inspected individually to ensure that the sequence similarity extended over most of their lengths. This curatorial process resulted in the identification of more than 100 gene families, half of which have no database matches. The largest identified gene family (16 members: MJ0625, MJECL28, MJ1076, MJ1006, MJ1659, MJ0075, MJ1609, MJECL19, MJECL18, MJ0147, MJ0801, MJ1301, MJ0632, MJ1010, MJ0074, and MJ0439) contains almost 1% of the total predicted protein-coding regions in *M. jannaschii*.

Despite the availability for comparison of two complete bacterial genomes and several hundred megabase pairs of eukaryotic sequence data, the majority of genes in *M. jannaschii* cannot be identified on the basis of sequence similarity. Previous evidence for the shared common ancestry of the Archaeal and Eukaryotic was based on a small set gene sequences (Iwabe, N., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9355 (1989); Gogarten J.P., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:6661 (1989); Brown, J.R. and Doolittle, W.F., *Proc. Natl. Acad. Sci. USA* 92:2441 (1995)). The complete genome of *M. jannaschii* allows us to move beyond a "gene by gene" approach to one that encompasses the larger picture of metabolic capacity and cellular systems. The anabolic genes of *M. jannaschii* (especially those related to energy production and nitrogen fixation) reveal an ancient metabolic world shared largely by Bacteria and Archaea. That many basic autotrophic pathways appear to have a common evolutionary origin suggests that the most recent universal common ancestor to all three domains of extant life had the capacity for autotrophy. The Archaea and Bacteria also share structural and organizational features that the most recent universal prokaryotic ancestors also likely possessed, such as circular genomes and genes organized as operons. In contrast, the cellular information-processing and secretion systems in *M. jannaschii* demonstrate the common ancestry of Eukaryotes and Archaea. Although there are components of these systems are present in all three domains, their apparent refinement over time—especially transcription and translation—indicate that the Archaea and Eukaryotes share a common evolutionary trajectory independent of the lineage of Bacteria.

## Example 2

### *Preparation of PCR Primers and Amplification of DNA*

Various fragments of the *Methanococcus jannaschii* genome, such as those disclosed in Tables 2(a), 2(b) and 3 can be used, in accordance with the present invention, to prepare PCR primers. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. The PCR primers are useful during PCR cloning of the ORFs described herein.

## Example 3

### *Gene expression from DNA Sequences Corresponding to ORFs*

A fragment of the *Methanococcus jannaschii* genome (preferably, a protein-encoding sequence) provided in Tables 2(a), 2(b) or 3 is introduced into an expression vector using conventional technology (techniques to transfer cloned sequences into expression vectors that direct protein translation in mammalian, yeast, insect or bacterial expression systems are well known in the art). Commercially available vectors and expression systems are available from a variety of suppliers including Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield *et al.*, U.S. Pat. No. 5,082,767, which is hereby incorporated by reference.

The following is provided as one exemplary method to generate polypeptide(s) from a cloned ORF of the *Methanococcus* genome whose sequence is provided in SEQ ID NOS: 1, 2 and 3. A poly A sequence can be

added to the construct by, for example, splicing out the poly A sequence from pSG5 (Stratagene) using *Bgl*I and *Sal*I restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene) for use in eukaryotic expression systems. pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene. The *Methanococcus* DNA is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the *Methanococcus* DNA and containing restriction endonuclease sequences for *Pst*I incorporated into the 5' primer and *Bgl*II at the 5' end of the corresponding *Methanococcus* DNA 3' primer, taking care to ensure that the *Methanococcus* DNA is positioned such that its followed with the poly A sequence. The purified fragment obtained from the resulting PCR reaction is digested with *Pst*I, blunt ended with an exonuclease, digested with *Bgl*II, purified and ligated to pXT1, now containing a poly A sequence and digested *Bgl*II.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Missouri). The protein is preferably released into the supernatant. However if the protein has membrane binding domains, the protein may additionally be retained within the cell or expression may be restricted to the cell surface.

Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted *Methanococcus* DNA sequence are injected into mice to generate antibody to the polypeptide encoded by the *Methanococcus* DNA.

If antibody production is not possible, the *Methanococcus* DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example,  $\beta$ -globin. Antibody to  $\beta$ -globin is used to purify the chimeric. Corresponding protease cleavage sites engineered between the  $\beta$ -globin

gene and the *Methanococcus* DNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating  $\beta$ -globin chimerics is pSG5 (Stratagene). This vector encodes rabbit  $\beta$ -globin. Intron II of the rabbit  $\beta$ -globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are available from the technical assistance representatives from Stratagene, Life Technologies, Inc., or Promega. Polypeptides may additionally be produced from either construct using in vitro translation systems such as In vitro Express™ Translation Kit (Stratagene).

#### **Example 4**

##### ***E. coli* Expression of a *M. jannaschii* ORF and protein purification**

A *M. jannaschii* ORF described in Table 2(a), 2(b), or 3 is selected and amplified using PCR oligonucleotide primers designed from the nucleotide sequences flanking the selected ORF and/or from portions of the ORF's NH<sub>2</sub>- or COOH-terminus. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5' and 3' sequences, respectively.

The restriction sites are selected to be convenient to restriction sites in the bacterial expression vector pD10 (pQE9), which is used for bacterial expression. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). [pD10]pQE9 encodes ampicillin antibiotic resistance ("Amp") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), a 6-His tag and restriction enzyme sites.

The amplified *M. jannaschii* DNA and the vector pQE9 both are digested with Sall and XbaI and the digested DNAs are then ligated together. Insertion of the *M. jannaschii* DNA into the restricted pQE9 vector places the *M. jannaschii* coding region downstream of and operably linked to the vector's IPTG-inducible

promoter and in-frame with an initiating AUG appropriately positioned for translation of the *M. jannaschii* protein.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook *et al.*,  
5 Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are  
10 suitable for expressing *M. jannaschii* protein, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis.  
15 Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm  
20 ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from *lac* repressor sensitive promoters, by inactivating the *lacI* repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified  
25 from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate-buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of  
30 chromatography to remove endotoxin followed by sterile filtration. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 µ/ml.

### Example 5

#### *Cloning and Expression of a M. jannaschii protein in a Baculovirus Expression System*

5 A *M. jannaschii* ORF described in Table 2(a), 2(b), or 3 is selected and amplified as above. The amplified DNA is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The DNA then is digested with XbaI and again purified on a 1% agarose gel. This DNA is designated herein as F2.

10 The vector pA2-GP is used to express the *M. jannaschii* protein in the baculovirus expression system as described in Summers *et al.*, A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). The pA2-GP expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient  
15 restriction sites. The signal peptide of AcMNPV gp67, including the N-terminal methionine, is located just upstream of a BamHI site. The polyadenylation site from the simian virus 40 ("SV40") is used for efficient polyadenylation. For an easy selection of recombinant virus, the beta-galactosidase gene from *E. coli* is inserted in the same orientation as the polyhedrin promoter and is followed by the  
20 polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

25 Many other baculovirus vectors could be used in place of pA2-GP, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al.*, *Virology* 170: 31-39, among others.



The plasmid is digested with the restriction enzyme XbaI and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V".

Fragment F2 and the dephosphorylated plasmid V2 are ligated together with T4 DNA ligase. *E. coli* HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the *M. jannaschii* gene by digesting DNA from individual colonies using XbaI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac*M. jannaschii*.

5 µg of the plasmid pBac*M. jannaschii* is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84: 7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac*M. jannaschii* are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained

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plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

5 Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200  $\mu$ l of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four  
10 days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. A clone containing properly inserted hESSB I, II and III is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as *V-M. jannaschii*.

15 Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus *V-M. jannaschii* at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine  
20 (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

## ***Example 6***

### ***Cloning and Expression in Mammalian Cells***

25 Most of the vectors used for the transient expression of a *M. jannaschii* gene in mammalian cells should carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g., COS cells) which

express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

5 A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein-coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, 10 e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular signals can also be used (e.g., human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 15 67109). Mammalian host cells that could be used include, human HeLa, 283, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells.

20 Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

25 The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al.*, *Biochem J.* 227:277-279 (1991); Bebbington *et al.*, *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are 30 selected. These cell lines contain the amplified gene(s) integrated into a

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chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al.*, *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al.*, *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

#### ***Example 6(a): Cloning and Expression in COS Cells***

The expression plasmid, pM. *jannaschii* HA, is made by cloning a cDNA encoding a *M. jannaschii* protein into the expression vector pcDNA1/Amp (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNA1/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

A DNA fragment encoding the *M. jannaschii* protein and an HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al.*, *Cell* 37:767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

5 The PCR amplified DNA fragment (generated as described above) and the vector, pcDNAI/Amp, are digested with HindIII and XhoI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the *M. jannaschii* protein-encoding fragment.

10 For expression of recombinant *M. jannaschii*, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of *M. jannaschii* protein by the vector.

15 Expression of the *M. jannaschii* HA fusion protein is detected by radiolabelling and immunoprecipitation, using methods described in, for example Harlow *et al.*, Antibodies: A Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing <sup>35</sup>S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

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**Example 6(b): Cloning and Expression in CHO Cells**

The vector pC1 is used for the expression of a *M. jannaschii* protein. Plasmid pC1 is a derivative of the plasmid pSV2-dhfr [ATCC Accession No. 37146]. Both plasmids contain the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F.W., Kellems, R.M., Bertino, J.R., and Schimke, R.T., 1978, J. Biol. Chem. 253:1357-1370, Hamlin, J.L. and Ma, C. 1990, Biochem. et Biophys. Acta, 1097:107-143, Page, M.J. and Sydenham, M.A. 1991, Biotechnology Vol. 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene it is usually co-amplified and over-expressed. It is state of the art to develop cell lines carrying more than 1,000 copies of the genes. Subsequently, when the methotrexate is withdrawn, cell lines contain the amplified gene integrated into the chromosome(s).

Plasmid pC1 contains for the expression of the gene of interest a strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, *et al.*, Molecular and Cellular Biology, March 1985:438-4470) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al.*, *Cell* 41:521-530, 1985). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, PvuII, and NruI. Behind these cloning sites the plasmid contains translational stop codons in all three reading frames followed by the 3' intron and the polyadenylation site of the rat preproinsulin gene. Other high efficient promoters can also be used for the expression, e.g., the human  $\beta$ -actin promoter, the SV40 early or late promoters or the long terminal

repeats from other retroviruses, e.g., HIV and HTLV. For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well.

5 Stable cell lines carrying the gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

10 The plasmid pC1 is digested with the restriction enzyme BamHI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

15 The *M. jannaschii* protein-encoding sequence is amplified using PCR oligonucleotide primers as described above. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., J. Mol. Biol. 196:947-950 (1987) is appropriately located in the vector portion of the construct. The amplified fragments are isolated from a 1% agarose gel as described above and then digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel.

20 The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 cells are then transformed and bacteria identified that contained the plasmid pC1 inserted in the correct orientation using the restriction enzyme BamHI. The sequence of the inserted gene is confirmed by DNA sequencing.

### ***Transfection of CHO-DHFR-cells***

25 Chinese hamster ovary cells lacking an active DHFR enzyme are used for transfection. 5 µg of the expression plasmid C1 are cotransfected with 0.5 µg of the plasmid pSVneo using the lipofecting method (Felgner *et al.*, *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the gene neo from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml

G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) and cultivated from 10-14 days. After this period, single clones are trypsinized and then seeded in 6-well petri dishes using different concentrations of methotrexate (25 nM, 50 nM, 100 nM, 200 nM, 400 nM).  
5 Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (500 nM, 1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M). The same procedure is repeated until clones grow at a concentration of 100  $\mu$ M.

The expression of the desired gene product is analyzed by Western blot  
10 analysis and SDS-PAGE.

### ***Example 7***

#### ***Production of an Antibody to a Methanococcus jannaschii Protein***

Substantially pure *M. jannaschii* protein or polypeptide is isolated from the transfected or transformed cells described above using an art-known method.  
15 The protein can also be chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

#### ***Monoclonal Antibody Production by Hybridoma Fusion***

20 Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., *Nature* 256:495 (1975) or modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The  
25 mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma



cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., *Meth. Enzymol.* 70:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989).

#### ***Polyclonal Antibody Production by Immunization***

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other molecules and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall (See Ouchterlony, O. *et al.*, Chap. 19 in: *Handbook of Experimental Immunology*, Wier, D., ed, Blackwell (1973)). Plateau

concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about  $12 \mu\text{M}$ ). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: *Manual of Clinical Immunology*, second edition, Rose and Friedman, (eds.), Amer. Soc. For Microbio., Washington, D.C. (1980).

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Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample.

Table 2A

Amino acid biosynthesis						
Aromatic amino acid family						
MJ1454	47830	48390	3-dehydroquininate dehydratase { <i>Escherichia coli</i> }	32.6	54.0	561
MJ0502	1029204	1027915	5-enolpyruvylshikimate 3-phosphate synthase { <i>Haemophilus influenzae</i> }	38.2	60.0	1290
MJ1075	456842	458158	anthranilate synthase, subunit I { <i>Clostridium thermocellum</i> }	52.7	72.1	1317
MJ0234	1247181	1246243	anthranilate synthase, subunit II' { <i>Thermotoga maritima</i> }	44.1	64.3	939
MJ0238	1242410	1241916	anthranilate synthase, subunit II'' { <i>Thermotoga maritima</i> }	52.6	75.0	495
MJ0246	1238364	1238660	chorismate mutase subunit A { <i>Erwinia herbicola</i> }	37.4	59.4	297
MJ0612	929781	928723	chorismate mutase subunit B { <i>Escherichia coli</i> }	33.2	56.2	1059
MJ1175	357469	358572	chorismate synthase { <i>Synechocystis</i> sp}	48.8	66.5	1104
MJ0918	621924	622682	indole-3-glycerol phosphate synthase { <i>Halobacterium volcanii</i> }	42.7	67.7	759
MJ0451	1068501	1067845	N-phosphoribosyl anthranilate isomerase { <i>Haloferax volcanii</i> }	41.9	62.5	657
MJ0637	904569	905264	prephenate dehydratase { <i>Lactococcus lactis</i> }	39.3	61.7	696
MJ1084	449533	448757	shikimate 5-dehydrogenase { <i>Escherichia coli</i> }	38.9	57.4	777
MJ1038	502619	501777	tryptophan synthase, subunit alpha { <i>Methanobacterium thermoautotrophicum</i> }	49.8	69.3	843
MJ1037	503929	502808	tryptophan synthase, subunit beta { <i>Acinetobacter calcoaceticus</i> }	62.2	78.7	1122

Aspartate family						
MJ1116	414120	415679	asparagine synthetase {Escherichia coli}	34.0	54.3	1560
MJ1056	476613	476170	asparagine synthetase {Bacillus subtilis}	33.0	54.6	444
MJ1391	132691	133833	aspartate aminotransferase {Sulfolobus solfataricus}	31.0	52.2	1143
MJ0684	859565	860632	aspartate aminotransferase {Sulfolobus solfataricus}	37.8	63.7	1068
MJ0001	1469369	1470142	aspartate aminotransferase {Sulfolobus solfataricus}	39.2	63.8	774
MJ0205	1273947	1274951	aspartate-semialdehyde dehydrogenase {Leptospira interrogans}	50.4	67.2	1005
MJ0571	963902	962544	aspartokinase I {Serratia marcescens}	37.0	56.7	1359
MJ1473	26812	27558	cobalamin-independent methionine synthase {Methanobacterium thermoautotrophicum}	47.7	65.3	747
MJ1097	433957	435159	diaminopimelate decarboxylase {Haemophilus influenzae}	43.2	66.6	1203
MJ1119	412913	412029	diaminopimelate epimerase {Haemophilus influenzae}	36.2	56.6	885
MJ0422	1090629	1091441	dihydrodipicolinate reductase {Haemophilus influenzae}	45.0	64.4	813
MJ0244	1239093	1239776	dihydrodipicolinate synthase {Haemophilus influenzae}	46.6	64.4	684
MJ1003	540278	539106	homoaconitase {Saccharomyces cerevisiae}	35.7	56.9	1173
MJ1602	1563296	1562289	homoserine dehydrogenase {Bacillus subtilis}	40.4	63.2	1008
MJ1104	427241	428128	homoserine kinase {Haemophilus influenzae}	30.1	53.9	888
MJ0020	1450056	1451210	L-asparaginase I {Haemophilus influenzae}	34.8	53.1	1155

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MJ0457	1064285	1063176	succinyl-diaminopimelate desuccinylase {Haemophilus influenzae}	27.0	45.8	1110
MJ1465	36982	38157	threonine synthase {Bacillus subtilis}	51.2	71.1	1176
<b>Glutamate family</b>						
MJ0069	1406333	1405455	acetylglutamate kinase {Bacillus stearothermophilus}	44.4	65.7	879
MJ0791	757315	758637	argininosuccinate lyase {Campylobacter jejuni}	41.3	65.6	1323
MJ0429	1087105	1086023	argininosuccinate synthase {Methanococcus vannielii}	70.2	86.8	1083
MJ0186	1287178	1288140	glutamate N-acetyltransferase {Bacillus stearothermophilus}	47.4	63.1	963
MJ1351	172535	174007	glutamate synthase (NADPH), subunit alpha {Escherichia coli}	40.5	54.0	1473
MJ1346	179417	178068	glutamine synthetase {Methanococcus voltae}	70.5	84.7	1350
MJ1096	435486	436508	N-acetyl-gamma-glutamyl-phosphate reductase {Bacillus subtilis}	40.4	63.6	1023
MJ0721	817148	816045	N-acetylornithine aminotransferase {Anabaena sp.}	46.7	67.0	1104
MJ0881	664952	665845	ornithine carbamoyltransferase {Halobacterium halobium}	43.0	69.6	894
<b>Pyruvate family</b>						
MJ0503	1027812	1026610	2-isopropylmalate synthase {Lactococcus lactis}	44.4	61.1	1203
MJ1392	131826	130633	2-isopropylmalate synthase {Anabaena sp.}	43.0	63.1	1194
MJ1271	256614	256216	3-isopropylmalate dehydratase {Salmonella typhimurium}	44.1	62.0	399
MJ1277	249421	249807	3-isopropylmalate dehydratase {Clostridium pasteurianum}	49.5	70.2	387
MJ0663	884580	883129	acetolactate synthase, large subunit {Porphyra umbilicalis}	34.5	54.6	1452
MJ0277	1207735	1209507	acetolactate synthase, large subunit {Bacillus subtilis}	50.2	69.7	1773

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MJ0161	1307199	1307702	acetolactate synthase, small subunit {Bacillus subtilis}	49.4	74.1	504
MJ1008	533323	534132	branched-chain amino acid aminotransferase {Escherichia coli}	42.6	59.0	810
MJ1276	250052	251710	dihydroxy-acid dehydratase {Lactococcus lactis}	44.6	65.1	1659
MJ1195	333450	335003	isopropylmalate synthase {Haemophilus influenzae}	42.9	63.7	1554
MJ1543	1615932	1614931	ketol-acid reductoisomerase {Bacillus subtilis}	53.7	77.0	1002
<b>Serine family</b>						
MJ1597	1568671	1567445	glycine hydroxymethyltransferase {Methanobacterium thermoautotrophicum}	69.8	80.7	1227
MJ1018	523454	524806	phosphoglycerate dehydrogenase {Bacillus subtilis}	42.7	65.4	1353
MJ1594	1571545	1571039	phosphoserine phosphatase {Haemophilus influenzae}	40.4	62.7	507
MJ0959	580672	581778	serine aminotransferase {Methanobacterium thermoformicum}	54.5	74.9	1107
<b>Histidine family</b>						
MJ1204	324063	324878	ATP phosphoribosyltransferase {Escherichia coli}	34.0	57.3	816
MJ1456	46532	45354	histidinol dehydrogenase {Lactococcus lactis}	47.6	67.5	1179
MJ0955	586179	585073	histidinol-phosphate aminotransferase {Bacillus subtilis}	37.7	60.8	1107
MJ0698	848921	848364	imidazoleglycerol-phosphate dehydrogenase {Methanobacterium thermoautotrophicum}	51.7	71.2	558
MJ0506	1024803	1025237	imidazoleglycerol-phosphate synthase (amidotransferase) {Lactococcus lactis}	45.6	62.1	435
MJ0411	1101451	1100636	imidazoleglycerol-phosphate synthase (cyclase) {Azospirillum brasilense}	61.5	78.8	816
MJ1430	71328	71047	phosphoribosyl-AMP cyclohydrolase {Methanococcus vannielii}	70.0	86.3	282

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MJ0302	1186990	1187208	phosphoribosyl-ATP pyrophosphohydrolase {Azotobacter chroococcum}	54.1	68.9	219
MJ1532	1628155	1627745	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase {Methanococcus thermolithotrophicus}	51.9	81.1	411
<b>Biosynthesis of cofactors, prosthetic groups, and carriers</b>						
MJ0603	937289	938566	glutamate-1-semialdehyde aminotransferase {Bacillus subtilis}	51.7	70.6	1278
MJ0569	966316	967137	porphobilinogen deaminase {Bacillus subtilis}	41.2	61.4	822
MJ0493	1035991	1036839	quinolinate phosphoribosyltransferase {Escherichia coli}	39.3	61.6	849
MJ0407	1105699	1104965	quinolinate synthetase {Cyanophora paradoxa}	37.2	58.8	735
MJ1388	136484	135309	S-adenosylhomocysteine hydrolase {Sulfolobus solfataricus}	61.7	78.5	1176
<b>Biotin</b>						
MJ1297	227704	227021	6-carboxyhexanoate-CoA ligase {Bacillus sphaericus}	42.2	62.2	684
MJ1298	227005	225890	8-amino-7-oxononanoate synthase {Bacillus sphaericus}	44.4	64.8	1116
MJ1300	225025	223709	adenosylmethionine-8-amino-7-oxononanoate aminotransferase {Bacillus sphaericus}	39.9	64.2	1317
MJ1619	1543130	1543552	bifunctional protein {Haemophilus influenzae}	25.7	54.9	423
MJ1296	228286	228843	biotin synthetase {Bacillus sphaericus}	38.2	62.5	558
MJ1299	225741	225100	dethiobiotin synthetase {Bacillus sphaericus}	37.0	59.0	642

Heme and porphyrin						
MJ1438	66330	65833	cobalamin (5'-phosphate) synthase {Escherichia coli}	26.1	48.7	498
MJ0552	983686	984417	cobalamin biosynthesis J protein {Salmonella typhimurium}	26.7	51.2	732
MJ1314	212528	211842	cobalamin biosynthesis protein D {Pseudomonas denitrificans}	38.0	61.0	687
MJ0022	1448163	1447273	cobalamin biosynthesis protein D {Salmonella typhimurium}	35.5	61.1	891
MJ1569	1592308	1591700	cobalamin biosynthesis protein M {Salmonella typhimurium}	29.5	54.7	609
MJ1091	442661	443239	cobalamin biosynthesis protein M {Salmonella typhimurium}	53.7	74.4	579
MJ0908	635150	631647	cobalamin biosynthesis protein N {Pseudomonas denitrificans}	37.5	57.6	3504
MJ0484	1046784	1045324	cobyrinic acid synthase {Methanococcus voltae}	73.7	89.8	1461
MJ1421	85381	86352	cobyrinic acid a,c-diamide synthase {Salmonella typhimurium}	32.1	55.0	972
MJ0143	1332080	1330965	glutamyl-tRNA reductase {Methanobacterium thermoautotrophicum}	47.8	66.9	1116
MJ0643	899800	898910	porphobilinogen synthase {Methanothermobacter sociabilis}	62.5	79.9	891
MJ0930	612059	611430	precorrin isomerase {Salmonella typhimurium}	38.7	62.0	630
MJ0771	780420	779932	precorrin-2 methyltransferase {Salmonella typhimurium}	30.4	55.9	489
MJ0813	734876	735547	precorrin-3 methylase {Salmonella typhimurium}	44.2	68.4	672
MJ1578	1583277	1582501	precorrin-3 methylase {Salmonella typhimurium}	54.6	76.5	777
MJ1522	1637017	1636385	precorrin-6Y methylase {Salmonella typhimurium}	30.6	52.3	633
MJ0391	1116729	1117202	precorrin-8W decarboxylase {Salmonella typhimurium}	23.9	49.1	474



MJ0965	573234	572509	uroporphyrin-III C-methyltransferase {Bacillus megaterium}	54.7	72.5	726
MJ0994	549022	549444	uroporphyrinogen III synthase {Bacillus subtilis}	27.8	49.4	423
<b>Menaquinone and ubiquinone</b>						
MJ1645	1509624	1508923	coenzyme PQQ synthesis protein III {Haemophilus influenzae}	32.2	53.3	702
<b>Molybdopterin</b>						
MJ0824	725986	726762	molybdenum cofactor biosynthesis moaA protein {Haemophilus influenzae}	30.0	57.3	777
MJ0167	1301836	1302162	molybdenum cofactor biosynthesis moaB protein {Escherichia coli}	46.4	69.6	327
MJ1135	396359	396781	molybdenum cofactor biosynthesis moaC protein {Haemophilus influenzae}	49.2	70.9	423
MJ0886	654158	656017	molybdenum cofactor biosynthesis moeA protein {Escherichia coli}	34.5	55.2	1860
MJ0666	879771	880943	molybdenum cofactor biosynthesis moeA protein {Haemophilus influenzae}	33.6	56.4	1173
MJ1663	1491265	1490831	molybdopterin-guanine dinucleotide biosynthesis protein A {Escherichia coli}	27.7	48.0	435
MJ1324	197777	197076	molybdopterin-guanine dinucleotide biosynthesis protein B {Escherichia coli}	32.2	57.7	702
<b>Pantothenate</b>						
MJ0913	626982	627779	pantothenate metabolism flavoprotein {Haemophilus influenzae}	34.1	55.7	798

<b>Riboflavin</b>					
MJ0055	1416688	1417278	GTP cyclohydrolase II {Bacillus subtilis}	35.8	56.0 591
MJ0671	874773	875396	riboflavin-specific deaminase {Actinobacillus pleuropneumoniae}	43.0	65.3 624
<b>Thioredoxin, glutaredoxin, and glutathione</b>					
MJ1536	1622694	1623533	thioredoxin reductase {Mycoplasma genitalium}	38.5	58.0 840
MJ0530	1005917	1005420	thioredoxin-2 {Saccharomyces cerevisiae}	33.0	63.3 498
MJ0307	1184114	1184332	thioredoxin/glutaredoxin {Methanobacterium thermoautotrophicum}	48.7	69.5 219
<b>Thiamine</b>					
MJ1026	514172	515440	thiamine biosynthesis protein {Bacillus subtilis}	45.0	66.1 1269
MJ0601	940113	939400	thiamine biosynthetic enzyme {Zea mays}	35.1	53.0 714
<b>Pyridine nucleotides</b>					
MJ1352	170567	171163	NH(3)-dependent NAD+ synthetase {Mycoplasma genitalium}	47.5	63.8 597
<b>Cell envelope</b>					
<b>Membranes, lipoproteins, and porins</b>					
MJ0544	989805	990443	dolichyl-phosphate mannosyl synthase {Trypanosoma brucei}	35.1	57.1 639
MJ1057	475508	474981	glycosyl transferase {Neisseria gonorrhoeae}	25.8	50.0 528
MJ0611	931098	930679	membrane protein {Saccharum sp.}	50.0	57.2 420
MJ0827	724322	723900	membrane protein {Homo sapiens}	44.9	67.0 423

Murein sacculus and peptidoglycan						
MJ1160	371691	370390	amidase {Moraxella catarrhalis}	24.6	36.1	1302
MJ0204	1276277	1275219	amidophosphoribosyltransferase {Bacillus subtilis}	52.0	72.9	1059
Surface polysaccharides, lipopolysaccharides and antigens						
MJ0924	617598	618035	capsular polysaccharide biosynthesis protein {Staphylococcus aureus}	31.3	46.9	438
MJ1061	469649	470293	capsular polysaccharide biosynthesis protein D {Staphylococcus aureus}	56.3	72.2	645
MJ1055	478643	477735	capsular polysaccharide biosynthesis protein I {Staphylococcus aureus}	50.7	74.4	909
MJ1059	472326	471904	capsular polysaccharide biosynthesis protein M {Staphylococcus aureus}	34.4	55.0	423
MJ1607	1555624	1554455	LPS biosynthesis related rfbu-protein {Haemophilus influenzae}	33.4	57.6	1170
MJ1113	417528	418352	N-acetylglucosamine-1-phosphate transferase {Sulfolobus acidocaldarius}	29.9	57.9	825
MJ0399	1110873	1112204	phosphomannomutase {Vibrio cholerae}	37.0	57.8	1332
MJ1068	462901	464265	putative O-antigen transporter {Shigella flexneri}	24.5	46.6	1365
MJ1066	464369	465430	spore coat polysaccharide biosynthesis protein C {Bacillus subtilis}	55.3	75.8	1062
MJ1065	465444	466454	spore coat polysaccharide biosynthesis protein E {Bacillus subtilis}	37.9	59.0	1011
MJ1063	467331	467828	spore coat polysaccharide biosynthesis protein F {Bacillus subtilis}	36.0	55.4	498
MJ1062	467870	469279	spore coat polysaccharide biosynthesis protein G {Bacillus subtilis}	32.0	54.5	1410
MJ0211	1269601	1268732	UDP-glucose 4-epimerase {Streptococcus thermophilus}	35.1	54.8	870
MJ1054	481027	478712	UDP-glucose dehydrogenase {Xanthomonas campestris}	42.8	63.4	2316
MJ0428	1087456	1088655	UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase {Escherichia coli}	45.1	68.2	1200

Surface structures						
MJ0891	650616	650005	flagellin B1 {Methanococcus voltae}	55.4	71.6	612
MJ0892	649880	649269	flagellin B2 {Methanococcus voltae}	61.1	78.4	612
MJ0893	649163	648516	flagellin B3 {Methanococcus voltae}	59.1	78.7	648
Cellular processes						
Cell division						
MJ1489	10595	8721	cell division control protein {Saccharomyces cerevisiae}	34.8	57.7	1875
MJ0363	1142460	1140220	cell division control protein 21 {Schizosaccharomyces pombe}	30.0	51.4	2241
MJ1156	375317	377947	cell division control protein CDC48 {Saccharomyces cerevisiae}	51.9	71.7	2631
MJ0169	1300988	1300329	cell division inhibitor {Bacillus subtilis}	28.8	51.2	660
MJ0579	957291	958088	cell division inhibitor {Bacillus subtilis}	31.8	53.2	798
MJ0547	988025	988732	cell division inhibitor {Bacillus subtilis}	32.8	57.7	708
MJ0084	1393471	1392869	cell division inhibitor minD {Escherichia coli}	32.1	50.4	603
MJ0174	1295971	1294976	cell division protein {Drosophila melanogaster}	28.4	54.6	996
MJ0370	1135876	1134956	cell division protein fisZ {Anabaena 7120}	50.7	71.7	921
MJ1376	147975	147343	cell division protein J {Haemophilus influenzae}	39.8	58.5	633
MJ0622	920029	921168	cell division protein Z {Haloflex volcanii}	51.0	71.7	1140
MJ0148	1326798	1327538	centromere/microtubule-binding protein {Saccharomyces cerevisiae}	42.7	64.7	741

MJ1647	1508164	1507907	DNA binding protein {Methanococcus voltae}	54.7	80.3	258
MJ1643	1513857	1510351	P115 protein {Mycoplasma hyorhinis}	30.3	55.4	3507
<b>Chaperones</b>						
MJ0999	543921	545471	chaperonin {Methanopyrus kandleri}	73.5	87.6	1551
MJ0285	1202058	1202459	heat shock protein {Clostridium acetobutylicum}	29.0	44.6	402
MJ0278	1207276	1207548	rotamase, peptidyl-prolyl cis-trans isomerase {Haemophilus influenzae}	40.7	60.5	273
MJ0825	725091	725765	rotamase, peptidyl-prolyl cis-trans isomerase {Pseudomonas fluorescens}	31.8	60.8	675
<b>Detoxification</b>						
MJ0736	804803	805453	alkyl hydroperoxide reductase {Sulfolobus solfataricus}	66.1	84.8	651
MJ1541	1618786	1619868	N-ethylamine chlorohydrolase {Rhodococcus rubropertinctus}	29.2	56.3	1083
<b>Protein and peptide secretion</b>						
MJ0478	1051985	1050678	preprotein translocase secY {Methanococcus vannielii}	70.9	88.8	1308
MJ0111	1365253	1364216	protein-export membrane protein {Streptomyces coelicolor}	25.9	51.7	1038
MJ1253	276673	277377	protein-export membrane protein {Escherichia coli}	30.5	57.0	705
MJ0260	1226090	1226644	signal peptidase {Canis familiaris}	32.6	54.5	555
MJ0101	1376106	1377308	signal recognition particle protein {Haemophilus influenzae}	42.0	61.6	1203
MJ0291	1198470	1197244	signal recognition particle protein {Sulfolobus acidocaldarius}	48.3	69.4	1227

Transformation						
MJ0781	768702	770798	k1bA protein {Plasmid RK2}	34.6	54.9	2097
MJ0940	602402	601929	transformation sensitive protein {Homo sapiens}	35.0	53.9	474
Cellular processes						
MJECL17	20110	19889	archaeal histone {Pyrococcus sp.}	58.8	81.0	221
MJECL29	36456	26220	archaeal histone {Pyrococcus sp.}	64.2	83.6	236
MJ1258	271686	271486	archaeal histone {Pyrococcus sp.}	71.7	83.6	201
MJ0168	1301348	1301548	archaeal histone {Pyrococcus sp.}	67.2	86.6	201
MJ0932	610153	609953	archaeal histone {Pyrococcus sp.}	67.2	86.6	201
Central intermediary metabolism						
Amino sugars						
MJ1420	90244	86939	glutamine--fructose-6-phosphate transaminase {Escherichia coli}	41.2	61.5	3306
Degradation of polysaccharides						
MJ1611	1550816	1549542	alpha-amylase {Pyrococcus furiosus}	27.0	50.5	1275
MJ0555	981500	980529	endoglucanase {Homo sapiens}	44.1	66.8	972
MJ1610	1551992	1550967	glucoamylase {Clostridium sp}	28.0	49.2	1026

Other						
MJ1656	1498675	1497965	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase {Escherichia coli}	40.2	61.6	711
MJ0406	1106800	1105907	ribokinase {Escherichia coli}	23.2	46.3	894
MJ0309	1182259	1183077	ureohydrolase {Methanothermus fervidus}	40.9	60.7	819
Phosphorus compounds						
MJ0963	575418	577049	N-methylhydantoinase {Arthrobacter sp.}	32.6	53.0	1632
MJ0964	573516	575345	N-methylhydantoinase {Arthrobacter sp.}	37.7	56.4	1830
Polyamine biosynthesis						
MJ0535	1001006	1002031	acetylputrescine aminohydrolase {D01044 Mycoplasma}	33.3	48.6	1026
MJ0313	1179250	1179801	spermidine synthase {Homo sapiens}	32.3	57.7	552
Polysaccharides-(cytoplasmic)						
MJ1606	1555858	1557354	glycogen synthase {Hordeum vulgare}	33.7	58.3	1497
Nitrogen metabolism						
MJ1187	345237	344335	ADP-ribosylglycohydrolase (draG) {Rhodospirillum rubrum}	29.8	50.8	903
MJ0713	824113	826278	hydrogenase accessory protein {Azotobacter chroococcum}	33.8	54.8	2166
MJ0214	1267658	1267314	hydrogenase accessory protein {Azotobacter chroococcum}	30.7	56.5	345
MJ0676	869311	870276	hydrogenase expression/formation protein {Rhizobium leguminosarum}	46.1	65.3	966
MJ0442	1075480	1076028	hydrogenase expression/formation protein B {Rhizobium leguminosarum}	44.6	64.0	549
MJ0200	1279494	1279739	hydrogenase expression/formation protein C {Azotobacter vinelandii}	40.0	68.8	246

MJ0993	549539	550525	hydrogenase expression/formation protein D {Alcaligenes eutrophus}	44.7	63.5	987
MJ0631	914544	914089	hydrogenase maturation protease {Escherichia coli}	33.9	58.9	456
MJ1093	441468	440584	nifB protein {Anabaena sp}	43.1	67.2	885
MJ0879	667622	666984	nitrogenase reductase {Methanococcus voltae}	77.2	89.1	639
MJ0685	859442	858696	nitrogenase reductase related protein {Clostridium pasteurianum}	31.7	49.6	747
MJ1051	483344	484411	nodulation factor production protein {Bradyrhizobium japonicum}	32.1	51.1	1068
MJ1058	473947	473141	nodulation factor production protein {Bradyrhizobium japonicum}	37.7	58.0	807
<b>Carbon Fixation</b>						
MJ0152	1325036	1322820	carbon monoxide dehydrogenase, alpha subunit {Clostridium thermoaceticum}	42.1	65.6	2217
MJ0153	1322553	1320256	carbon monoxide dehydrogenase, alpha subunit {Methanotherix soehngenii}	47.9	67.3	2298
MJ0156	1319256	1317883	carbon monoxide dehydrogenase, alpha subunit {Clostridium thermoaceticum}	47.8	69.5	1374
MJ0728	809951	811783	carbon monoxide dehydrogenase, beta subunit {Rhodospirillum rubrum}	35.9	55.0	1833
MJ0112	1362285	1363667	corrinoid/iron-sulfur protein, large subunit {Clostridium thermoaceticum}	32.9	55.1	1383
MJ0113	1361128	1362030	corrinoid/iron-sulfur protein, small subunit {Clostridium thermoaceticum}	37.7	58.8	903
MJ1235	292453	293673	ribulose biphosphate carboxylase, large subunit {Synechococcus sp}	42.4	60.3	1221



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Energy metabolism						
<b>Aerobic</b>						
MJ0649	896262	894919	NADH oxidase {Enterococcus faecalis}	28.0	50.4	1344
MJ0520	1011104	1011892	NADH-ubiquinone oxidoreductase, subunit I {Paracentrotus lividus}	29.5	53.9	789
<b>Anaerobic</b>						
MJ0092	1385748	1384282	fumarate reductase {Thermoplasma acidophilum}	40.2	57.0	1467
<b>ATP-proton motive force interconversion</b>						
MJ0217	1263468	1265171	ATP synthase, subunit A {Enterococcus hirae}	60.3	76.6	1704
MJ0216	1265356	1266615	ATP synthase, subunit B {Methanosarcina barkeri}	69.4	84.5	1260
MJ0219	1261985	1263040	ATP synthase, subunit C {Haloflex volcanii}	28.1	50.0	1056
MJ0615	926124	926663	ATP synthase, subunit D {Enterococcus hirae}	34.8	56.8	540
MJ0220	1261297	1261737	ATP synthase, subunit E {Methanosarcina mazei}	29.0	50.0	441
MJ0218	1263054	1263347	ATP synthase, subunit F {Haloflex volcanii}	21.5	52.1	294
MJ0222	1258252	1260294	ATP synthase, subunit I {Enterococcus hirae}	27.6	52.2	2043
MJ0221	1260641	1261060	ATP synthase, subunit K {Enterococcus hirae}	34.6	59.8	420

Electron transport						
MJ1446	57416	56646	cytochrome-c3 hydrogenase, gamma chain {Pyrococcus furiosus}	40.1	52.4	771
MJ0741	803000	803320	desulfoferrodoxin {Desulfovibrio vulgaris}	44.0	59.4	321
MJ0578	958094	958900	ferredoxin {Clostridium sticklandii}	49.1	56.9	807
MJ0061	1411998	1411759	ferredoxin {Methanococcus thermolithotrophicus}	42.9	59.0	240
MJ0722	815808	816038	ferredoxin {Methanobacterium thermoautotrophicum}	42.3	60.6	231
MJ0099	1379076	1379456	ferredoxin {Desulfovibrio desulfuricans}	40.0	62.0	381
MJ0199	1279976	1279791	ferredoxin {Methanococcus thermolithotrophicus}	74.6	84.8	186
MJ0533	1003408	1003575	ferredoxin 2[4Fe-4S] homolog {Methanosarcina thermophila}	36.9	54.4	168
MJ0624	918981	918808	ferredoxin 2[4Fe-4S] {Methanosarcina thermophila}	48.0	68.0	174
MJ0267	1217567	1218463	ferredoxin oxidoreductase, alpha subunit {Klebsiella pneumoniae}	29.4	50.2	897
MJ0276	1209645	1210727	ferredoxin oxidoreductase, alpha subunit {Halobacterium halobium}	44.5	63.0	1083
MJ0266	1218644	1219387	ferredoxin oxidoreductase, beta subunit {Klebsiella pneumoniae}	32.6	51.0	744
MJ0537	998693	999424	ferredoxin oxidoreductase, beta subunit {Halobacterium halobium}	41.3	61.1	732
MJ0268	1217015	1217272	ferredoxin oxidoreductase, delta subunit {Pyrococcus furiosus}	58.9	71.8	258
MJ0536	999441	999980	ferredoxin oxidoreductase, gamma subunit {Pyrococcus furiosus}	32.0	50.9	540
MJ0269	1216601	1216993	ferredoxin oxidoreductase, gamma subunit {Pyrococcus furiosus}	55.6	74.7	393
MJ0732	806970	808100	flavoprotein {Methanobacterium thermoautotrophicum}	40.4	62.3	1131
MJ1192	339066	338095	methylviologen-reducing hydrogenase, alpha chain {Methanococcus voltae}	75.0	88.6	972

MJ1191	340221	339385	methylyliogen-reducing hydrogenase, gamma chain {Methanococcus voltae}	71.5	83.3	837
MJ1362	160414	161055	NADH dehydrogenase, subunit I {Mitochondrion Oncorhynchus}	23.1	50.0	642
MJ0514	1016474	1017223	polyferredoxin {Methanococcus voltae}	36.7	52.5	750
MJ0934	608147	607521	polyferredoxin {Methanothermus fervidus}	40.9	54.3	627
MJ1303	220214	221701	polyferredoxin {Methanobacterium thermoautotrophicum}	39.5	56.1	1488
MJ1193	337655	336591	polyferredoxin {Methanococcus voltae}	61.7	74.5	1065
MJ1227	301853	301257	pyruvate formate-lyase activating enzyme {Clostridium pasteurianum}	31.4	50.0	597
MJ0735	805546	805785	rubredoxin {Clostridium thermosaccharolyticum}	59.7	77.0	240
MJ0740	803522	803659'	rubredoxin {Clostridium thermosaccharolyticum}	64.5	84.5	138
<b>Fermentation</b>						
MJ0007	1463447	1462359	2-hydroxyglutaryl-CoA dehydratase, subunit beta {Acidaminococcus fermentans}	22.6	48.2	1089
<b>Gluconeogenesis</b>						
MJ1479	22527	21358	alanine aminotransferase 2 {Panicum miliaceum}	30.1	50.0	1170
MJ0542	991264	994794	phosphoenolpyruvate synthase {Pyrococcus furiosus}	60.3	78.3	3531

Glycolysis						
MJ1482	18946	18044	2-phosphoglycerate kinase {Methanothermus fervidus}	47.1	70.9	903
MJ0641	901393	902325	3-phosphoglycerate kinase {Methanothermus fervidus}	58.2	78.1	933
MJ0232	1248239	1249432	enolase {Bacillus subtilis}	57.7	78.2	1194
MJ1605	1557395	1558597	glucose-6-phosphate isomerase {Bacillus stearothermophilus}	32.3	54.6	1203
MJ1146	386093	387055	glyceraldehyde 3-phosphate dehydrogenase {Methanothermus fervidus}	59.5	77.6	963
MJ0490	1038560	1037697	lactate dehydrogenase {Thermotoga maritima}	39.9	63.2	864
MJ1411	100555	99167	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase {L15191 Streptococcus}	39.2	59.6	1389
MJ0108	1367951	1366716	pyruvate kinase {Bacillus stearothermophilus}	39.1	60.5	1236
MJ1528	1631071	1631589	triosephosphate isomerase {Mycoplasma genitalium}	29.0	49.1	519
Pentose phosphate pathway						
MJ0680	865484	866083	pentose-5-phosphate-3-epimerase {Solanum tuberosum}	44.2	62.5	600
MJ1603	1560724	1560047	ribose 5-phosphate isomerase {Mus musculus}	42.0	63.4	678
MJ0960	580121	580576	transaldolase {Bacillus subtilis}	60.7	79.5	456
MJ0681	864603	865355	transketolase' {Homo sapiens}	43.7	58.5	753
MJ0679	866375	867073	transketolase" {Homo sapiens}	36.0	61.3	699

Pyruvate dehydrogenase						
MJ0636	906464	905292	dihydrolipoamide dehydrogenase {Haloflex volcanii}	28.9	51.0	1173
Sugars						
MJ1418	91211	90669	fucose-1-phosphate aldolase {Haemophilus influenzae}	29.1	48.7	543
TCA cycle						
MJ0499	1031331	1032530	aconitase {Saccharomyces cerevisiae}	29.7	49.8	1200
MJ1294	229770	230381	fumarate hydratase, class I' {Bacillus stearothermophilus}	35.1	55.7	612
MJ0617	925239	924778	fumarate hydratase, class I'' {Bacillus stearothermophilus}	43.8	66.0	462
MJ1596	1568967	1569998	isocitrate dehydrogenase {Thermus aquaticus}	42.9	61.4	1032
MJ0720	817433	818431	isocitrate dehydrogenase (NADP) {Thermus aquaticus}	48.0	64.7	999
MJ1425	77051	76299	malate dehydrogenase {Methanothermobacter fervidus}	61.3	77.6	753
MJ0033	1438609	1437116	succinate dehydrogenase, flavoprotein subunit {Escherichia coli}	41.8	58.1	1494
MJ1246	282664	283449	succinyl-CoA synthetase, alpha subunit {Escherichia coli}	59.6	74.8	786
MJ0210	1271318	1270227	succinyl-CoA synthetase, beta subunit {Thermus aquaticus}	48.8	68.7	1092

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Methanogenesis						
MJ0253	1232773	1232405	8-hydroxy-5-deazaflavin-reducing hydrogenase, delta subunit {Methanobacterium thermoautotrophicum}	47.1	71.0	369
MJ1035	505234	506022	coenzyme F420-dependent N5,N10-methylene-tetrahydromethanopterin dehydrogenase {Methanobacterium thermoautotrophicum}	66.5	79.8	789
MJ0727	811895	812725	coenzyme F420-reducing hydrogenase, alpha subunit {Methanobacterium thermoautotrophicum}	26.8	45.8	831
MJ0029	1442517	1441279	coenzyme F420-reducing hydrogenase, alpha subunit {Methanococcus voltae}	50.3	66.1	1239
MJ0030	1441022	1440558	coenzyme F420-reducing hydrogenase, alpha subunit {Methanococcus voltae}	66.5	83.3	465
MJ1349	175566	176222	coenzyme F420-reducing hydrogenase, beta subunit {Methanococcus voltae}	36.6	55.7	657
MJ0725	813779	814453	coenzyme F420-reducing hydrogenase, beta subunit {Methanobacterium thermoautotrophicum}	41.0	62.0	675
MJ0870	677657	679372	coenzyme F420-reducing hydrogenase, beta subunit {Methanobacterium thermoautotrophicum}	42.7	63.2	1716
MJ0032	1439835	1438990	coenzyme F420-reducing hydrogenase, beta subunit {Methanococcus voltae}	72.0	85.5	846
MJ0726	812987	813499	coenzyme F420-reducing hydrogenase, gamma subunit {Methanococcus voltae}	42.7	59.4	513
MJ0031	1440505	1439873	coenzyme F420-reducing hydrogenase, gamma subunit {Methanococcus voltae}	75.5	87.3	633
MJ0295	1192687	1193304	formate dehydrogenase (fdhD) {Wolinella succinogenes}	35.6	57.7	618
MJ0006	1463887	1465020	formate dehydrogenase, alpha subunit {Methanobacterium formicicum}	41.6	61.1	1134
MJ1353	168767	170344	formate dehydrogenase, alpha subunit {Methanobacterium formicicum}	54.2	70.9	1578
MJ0005	1465405	1466247	formate dehydrogenase, beta subunit {Methanobacterium formicicum}	49.5	72.1	843

MJ0155	1319767	1319315	formate dehydrogenase, iron-sulfur subunit {Wolinella succinogenes}	41.7	56.9	453
MJ0264	1220122	1220433	formate hydrogenlyase, subunit 2 {Escherichia coli}	42.9	59.8	312
MJ0265	1219502	1219930	formate hydrogenlyase, subunit 2 {Escherichia coli}	45.5	61.0	429
MJ0515	1013710	1014735	formate hydrogenlyase, subunit 5 {Escherichia coli}	31.0	51.1	1026
MJ1027	514001	512871	formate hydrogenlyase, subunit 5 {Escherichia coli}	34.3	53.3	1131
MJ1363	159614	160018	formate hydrogenlyase, subunit 7 {Escherichia coli}	38.4	60.9	405
MJ0516	1013157	1013600	formate hydrogenlyase, subunit 7 {Escherichia coli}	48.8	65.6	444
MJ0318	1175065	1175823	formylmethanofuran:tetrahydromethanopterin formyltransferase {Methanobacterium thermoautotrophicum}	68.6	84.5	759
MJ1338	185930	185007	H(2)-dependent methylenetetrahydromethanopterin dehydrogenase related protein {Methanobacterium thermoautotrophicum}	29.1	50.5	924
MJ0715	823334	822423	H2-forming N5,N10-methylene-tetrahydromethanopterin dehydrogenase-related protein {Methanococcus voltae}	29.9	52.5	912
MJ0784	765279	764272	H2-forming N5,N10-methylene-tetrahydromethanopterin dehydrogenase {Methanococcus voltae}	73.6	85.5	1008
MJ1190	342199	341003	heterodisulfide reductase, subunit A {Methanobacterium thermoautotrophicum}	58.0	75.2	1197
MJ0743	801736	802422	heterodisulfide reductase, subunit B {Methanobacterium thermoautotrophicum}	59.3	79.0	687
MJ0863	684944	685798	heterodisulfide reductase, subunit B {Methanobacterium thermoautotrophicum}	63.2	80.2	855
MJ0744	801103	801489	heterodisulfide reductase, subunit C {Methanobacterium thermoautotrophicum}	53.4	68.4	387
MJ0864	684283	684840	heterodisulfide reductase, subunit C {Methanobacterium thermoautotrophicum}	52.6	69.9	558
MJ0118	1357167	1356667	methyl coenzyme M reductase II operon, protein D {Methanothermobacter ferredoxinus}	53.2	77.5	501

MJ0083	1395319	1393880	methyl coenzyme M reductase II, alpha subunit {Methanothermus fervidus}	89.8	95.5	1440
MJ0081	1397700	1396351	methyl coenzyme M reductase II, beta subunit {Methanothermus fervidus}	79.7	89.4	1350
MJ0082	1396335	1395538	methyl coenzyme M reductase II, gamma subunit {Methanothermus fervidus}	83.0	92.1	798
MJ0844	702037	701465	methyl coenzyme M reductase operon, protein C {Methanococcus vannielii}	82.5	92.6	573
MJ0843	702395	702069	methyl coenzyme M reductase operon, protein D {Methanococcus voltae}	58.0	81.4	327
MJ1662	1491537	1493201	methyl coenzyme M reductase system, component A2 {Methanobacterium thermoautotrophicum}	37.1	60.1	1665
MJ1242	284878	286338	methyl coenzyme M reductase system, component A2 {Methanobacterium thermoautotrophicum}	60.9	77.8	1461
MJ0846	700322	698880	methyl coenzyme M reductase, alpha subunit {Methanococcus voltae}	86.1	92.1	1443
MJ0842	703907	702576	methyl coenzyme M reductase, beta subunit {Methanococcus vannielii}	75.3	87.4	1332
MJ0845	701389	700673	methyl coenzyme M reductase, gamma subunit {Methanococcus vannielii}	78.7	91.3	717
MJ1636	1520054	1519128	N5,N10-methenyl-tetrahydromethanopterin cyclohydrolase {Methanobacterium thermoautotrophicum}	69.6	82.3	927
MJ1534	1625526	1624534	N5,N10-methylene tetrahydromethanopterin reductase {Methanobacterium thermoautotrophicum}	66.2	79.7	993
MJ0850	696203	695895	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	36.6	59.8	309
MJ0849	696884	696216	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	41.8	62.3	669
MJ0852	695117	694914	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	37.1	64.6	204



MJ0851	695866	695138	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	55.2	73.5	729
MJ0847	698519	697749	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	58.3	76.4	771
MJ0854	694607	693651	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	62.1	77.5	957
MJ0848	697696	697043	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase {Methanobacterium thermoautotrophicum}	63.5	77.8	654
MJ0853	694857	694639	N5-methyl-tetrahydromethanopterin:coenzyme M methyltransferase G {Methanobacterium thermoautotrophicum}	51.1	76.6	219
MJ1169	363822	362122	tungsten formylmethanofuran dehydrogenase, subunit A {Methanobacterium thermoautotrophicum}	69.4	81.5	1701
MJ1194	336096	335260	tungsten formylmethanofuran dehydrogenase, subunit B {Methanobacterium thermoautotrophicum}	71.1	84.0	837
MJ1171	361740	360973	tungsten formylmethanofuran dehydrogenase, subunit C {Methanobacterium thermoautotrophicum}	52.7	67.7	768
MJ0658	887575	886886	tungsten formylmethanofuran dehydrogenase, subunit C related protein {Methanobacterium thermoautotrophicum}	35.4	53.4	690
MJ1168	364202	363852	tungsten formylmethanofuran dehydrogenase, subunit D {Methanobacterium thermoautotrophicum}	55.2	74.8	351
MJ1165	366038	365637	tungsten formylmethanofuran dehydrogenase, subunit E {Methanobacterium thermoautotrophicum}	38.3	61.1	402
MJ1166	365484	364567	tungsten formylmethanofuran dehydrogenase, subunit F {Methanobacterium thermoautotrophicum}	47.6	67.4	918

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MJ1167	364516	364271	tungsten formylmethanofuran dehydrogenase, subunit G {Methanobacterium thermoautotrophicum}	43.1	58.5	246
<b>Fatty acid and phospholipid metabolism</b>						
MJ0705	840072	838927	3-hydroxy-3-methylglutaryl coenzyme A reductase {Haloflex volcanii}	49.8	67.3	1146
MJ1546	1612371	1611697	acyl carrier protein synthase {Pyrococcus furiosus}	63.1	78.0	675
MJ0860	688696	689499	bifunctional short chain isoprenyl diphosphate synthase {Methanobacterium thermoautotrophicum}	49.5	71.7	804
MJ1229	299478	300644	biotin carboxylase {Anabaena sp}	58.9	76.2	1167
MJ1212	316229	316786	CDP-diacylglycerol--serine O-phosphatidyltransferase {Bacillus subtilis}	45.5	63.7	558
MJ1504	1661217	1662188	lipopolysaccharide biosynthesis protein (bplD) {Bordetella pertussis}	44.3	63.1	972
MJ1087	446091	445231	melvalonate kinase {Schizosaccharomyces pombe}	31.5	53.7	861
MJ1549	1610772	1609735	nonspecific lipid-transfer protein {Pyrococcus furiosus}	46.9	66.0	1038
<b>Purines, pyrimidines, nucleosides, and nucleotides</b>						
<b>2'-Deoxyribonucleotide metabolism</b>						
MJ0832	719820	714604	anaerobic ribonucleoside-triphosphate reductase {Escherichia coli}	28.1	49.9	5217
MJ0430	1085497	1086009	deoxycytidine triphosphate deaminase {Desulfurolobus ambivalens}	40.4	61.5	513
MJ1102	429115	428648	deoxycytidine triphosphate deaminase, putative {Desulfurolobus ambivalens}	32.1	53.2	468
MJ0511	1019410	1020075	deoxyuridylate hydroxymethylase {Methanobacterium thermoautotrophicum}	39.4	59.6	666
MJ0937	606252	604921	glycinamide ribonucleotide synthetase {Homo sapiens}	37.1	55.0	1332

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Purine ribonucleotide biosynthesis						
MJ0929	613484	612135	adenylosuccinate lyase {Bacillus subtilis}	42.6	67.4	1350
MJ0561	976592	975741	adenylosuccinate synthetase {Haemophilus influenzae}	41.0	59.1	852
MJ1575	1586386	1585823	GMP synthetase {Borrelia burgdorferi}	41.4	66.7	564
MJ1131	399509	400264	GMP synthetase {Haemophilus influenzae}	52.0	72.3	756
MJ1616	1545605	1544271	inosine-5'-monophosphate dehydrogenase {Pyrococcus furiosus}	61.8	80.4	1335
MJ1265	262116	262436	nucleoside diphosphate kinase {Haemophilus influenzae}	51.5	68.3	321
MJ0616	925486	925941	phosphoribosylaminoimidazole carboxylase {Methanobrevibacter smithii}	56.3	76.2	456
MJ1592	1572482	1572009	phosphoribosylaminoimidazole succinocarboxamide synthase {Bacillus subtilis}	51.0	69.1	474
MJ0203	1277597	1276734	phosphoribosylformylglycinamide cyclo-ligase {Bacillus subtilis}	42.7	64.4	864
MJ1648	1507541	1507071	phosphoribosylformylglycinamide synthase I {Bacillus subtilis}	52.9	71.5	471
MJ1264	262585	264714	phosphoribosylformylglycinamide synthase II {Bacillus subtilis}	43.3	65.1	2130
MJ1486	13611	14633	phosphoribosylglycinamide formyltransferase 2 {Bacillus subtilis}	61.8	75.9	1023
MJ1366	155580	156431	ribose-phosphate pyrophosphokinase {Haemophilus influenzae}	34.1	55.5	852

Pyrimidine ribonucleotide biosynthesis						
MJ1581	1581578	1580661	aspartate carbamoyltransferase catalytic chain {Escherichia coli}	50.0	70.7	918
MJ1406	104548	104183	aspartate carbamoyltransferase regulatory chain {Escherichia coli}	39.1	65.1	366
MJ1378	145461	144037	carbamoyl-phosphate synthase, large chain {Bacillus subtilis}	59.7	80.0	1425
MJ1381	143097	141328	carbamoyl-phosphate synthase, pyrimidine-specific, large subunit {Bacillus caldolyticus}	54.7	75.7	1770
MJ1019	523003	522041	carbamoyl-phosphate synthase, small chain {Bacillus subtilis}	49.6	69.1	963
MJ1174	358774	360279	CTP synthase {Haemophilus influenzae}	56.7	74.0	1506
MJ0656	888785	888306	cytidylate kinase {Bacillus subtilis}	31.9	59.5	480
MJ1490	8032	6764	dihydroorotase {Bacillus caldolyticus}	34.5	56.3	1269
MJ0654	889442	890284	dihydroorotase dehydrogenase {Bacillus subtilis}	43.1	66.6	843
MJ0293	1196756	1196196	thymidylate kinase {Schizosaccharomyces pombe}	31.2	58.7	561
MJ1109	421875	421348	uridine 5'-monophosphate synthase {Dictyostelium discoideum}	38.4	64.6	528
MJ1259	271220	270543	uridylylate kinase {Haemophilus influenzae}	27.5	48.7	678

Salvage of nucleosides and nucleotides						
MJ1459	43987	42413	adenine deaminase {Bacillus subtilis}	35.9	61.7	1575
MJ1655	1499440	1499075	adenine phosphoribosyltransferase {Haemophilus influenzae}	35.8	62.5	366
MJ0060	1412894	1412139	methylothioadenosine phosphorylase {Homo sapiens}	41.3	63.2	756
MJ0667	879550	878150	thymidine phosphorylase {Mycoplasma genitalium}	30.5	52.2	1401
Sugar-nucleotide biosynthesis and conversions						
MJ1101	430386	429235	glucose-1-phosphate thymidyltransferase {Streptomyces griseus}	32.0	56.0	1152
MJ1334	188314	189084	UDP-glucose pyrophosphorylase {Mycoplasma genitalium}	42.7	63.6	771
Regulatory functions						
MJ0800	748410	747352	activator of (R)-2-hydroxyglutaryl-CoA dehydratase {Acidaminococcus fermentans}	31.8	51.2	1059
MJ0004	1466944	1466255	activator of (R)-2-hydroxyglutaryl-CoA dehydratase {Acidaminococcus fermentans}	39.0	61.1	690
MJ1344	180975	181229	nitrogen regulatory protein P-II {Haemophilus influenzae}	56.5	73.0	255
MJ0059	1413301	1413047	nitrogen regulatory protein P-II {Haemophilus influenzae}	56.5	75.3	255
MJ0300	1188832	1188194	putative transcriptional regulator {Bacillus subtilis}	27.8	50.3	639
MJ0151	1325766	1325323	putative transcriptional regulator {Pyrococcus furiosus}	51.0	65.0	444
MJ0723	815573	815190	putative transcriptional regulator {Pyrococcus furiosus}	51.2	82.3	384

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Replication						
Degradation of DNA						
MJ1434	68536	68048	endonuclease III {Bacillus subtilis}	28.7	58.1	489
MJ0613	927393	928424	endonuclease III {Bacillus subtilis}	41.3	66.3	1032
MJ1439	65786	65208	thermonuclease precursor {Staphylococcus hyicus}	36.8	64.1	579
DNA replication, restriction, modification, recombination, and repair						
MJ1029	510633	509875	dimethyladenosine transferase {Bacillus subtilis}	38.4	58.8	759
MJ0104	1373055	1371130	DNA helicase, putative {Homo sapiens}	35.2	56.7	1926
MJ0171	1297428	1299053	DNA ligase {Desulfurolobus ambivalens}	35.8	62.4	1626
MJ0869	680404	679445	DNA repair protein {Saccharomyces cerevisiae}	44.6	62.2	960
MJ1444	58945	58052	DNA repair protein RAD2 {Homo sapiens}	37.3	63.5	894
MJ0254	1232179	1231757	DNA repair protein RAD51 {Homo sapiens}	32.5	58.4	423
MJ0961	579580	577424	DNA replication initiator protein {Xenopus laevis}	28.1	40.0	2157
MJ1652	1503610	1501559	DNA topoisomerase I {Mycoplasma genitalium}	34.0	55.0	2052
MJ0885	656470	660960	DNA-dependent DNA polymerase family B {Pyrococcus sp.}	47.3	68.0	4491
MJ1529	1630880	1630413	methylated DNA protein cysteine methyltransferase {Haemophilus influenzae}	35.9	66.4	468
MJ1498	1548	715	modification methylase {Haemophilus parainfluenzae}	31.6	52.2	834
MJ0598	942522	941860	modification methylase {Haemophilus influenzae}	32.4	53.8	663
MJ1328	193775	192987	modification methylase {Haemophilus influenzae}	31.1	56.1	789

MJ0563	974521	975309	modification methylase {Methanobacterium thermoformicum}	34.7	56.2	789
MJ1200	326214	327248	modification methylase {Desulfovibrio desulfuricans}	39.7	56.7	1035
MJ0985	555045	555896	modification methylase {Methanobacterium thermoformicum}	54.5	73.0	852
MJ1149	383742	384248	mutator mutT protein {Escherichia coli}	40.3	63.9	507
MJ0942	600802	598916	probable ATP-dependent helicase {Haemophilus influenzae}	31.9	54.7	1887
MJ0247	1237945	1237322	proliferating-cell nuclear antigen {Saccharomyces cerevisiae}	31.5	54.3	624
MJ0026	1444598	1445224	proliferating-cell nucleolar antigen, 120 kDa {Homo sapiens}	48.1	66.1	627
MJ1422	79304	84727	replication factor C {Homo sapiens}	45.2	64.6	5424
MJ0884	662042	660969	replication factor C, large subunit {Homo sapiens}	32.5	49.2	1074
MJ1220	308420	310102	restriction modification enzyme, subunit M1 {Mycoplasma pulmonis}	32.9	54.4	1683
MJ0132	1345009	1345548	restriction modification enzyme, subunit M1 {Mycoplasma pulmonis}	37.3	61.1	540
MJ0130	1346511	1347179	restriction modification system S subunit {Spiroplasma citri}	29.3	59.2	669
MJ1512	1653580	1648742	reverse gyrase {Sulfolobus acidocaldarius}	41.8	62.4	4839
MJ0135	1341301	1341939	ribonuclease HII (mhB) {Escherichia coli}	45.2	64.6	639
MJECL42	55944	54271	type I restriction enzyme ECOR124/3 I M protein {Haemophilus influenzae}	39.7	61.4	1673
MJ0124	1349371	1352847	type I restriction enzyme {Haemophilus influenzae}	31.1	52.2	3477
MJ1214	313714	315828	type I restriction enzyme {Haemophilus influenzae}	29.5	52.2	2115
MJECL40	52581	49456	type I restriction enzyme {Haemophilus influenzae}	36.2	59.9	3125
MJ1531	1629137	1628493	type I restriction enzyme CfrI, specificity subunit {Citrobacter freundii}	38.4	57.9	645

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MJ1218	310547	311776	type I restriction-modification enzyme, S subunit { <i>Escherichia coli</i> }	29.7	49.7	1230
MJ0984	556397	555909	type II restriction enzyme { <i>Methanobacterium thermoformicicum</i> }	45.9	67.2	489
MJ0600	940932	940315	type II restriction enzyme DPNII { <i>Streptococcus pneumoniae</i> }	46.0	67.4	618
<b>Transcription</b>						
<b>DNA-dependent RNA polymerases</b>						
MJ1042	497715	493732	DNA-dependent RNA polymerase, subunit A' { <i>Methanococcus vannielii</i> }	74.5	88.1	3984
MJ1043	493546	491078	DNA-dependent RNA polymerase, subunit A'' { <i>Methanococcus vannielii</i> }	66.7	83.5	2469
MJ1041	499305	497866	DNA-dependent RNA polymerase, subunit B' { <i>Methanococcus vannielii</i> }	76.3	91.3	1440
MJ1040	501124	499862	DNA-dependent RNA polymerase, subunit B'' { <i>Methanococcus vannielii</i> }	72.7	87.4	1263
MJ0192	1283621	1283148	DNA-dependent RNA polymerase, subunit D { <i>Arabidopsis thaliana</i> }	39.5	58.6	474
MJ0397	1113901	1114371	DNA-dependent RNA polymerase, subunit E' { <i>Sulfolobus acidocaldarius</i> }	47.9	70.8	471
MJ0396	1114384	1114560	DNA-dependent RNA polymerase, subunit E'' { <i>Sulfolobus acidocaldarius</i> }	35.9	62.3	177
MJ1039	501599	501366	DNA-dependent RNA polymerase, subunit H { <i>Methanococcus vannielii</i> }	49.4	78.7	234
MJ1390	134111	134350	DNA-dependent RNA polymerase, subunit I { <i>Sulfolobus acidocaldarius</i> }	-0.9	-0.9	240
MJ0197	1281417	1281247	DNA-dependent RNA polymerase, subunit K { <i>Haloarcula marismortui</i> }	43.5	65.3	171
MJ0387	1119216	1119512	DNA-dependent RNA polymerase, subunit L { <i>Sulfolobus acidocaldarius</i> }	35.6	63.4	297
MJ0196	1281779	1281561	DNA-dependent RNA polymerase, subunit N { <i>Haloarcula marismortui</i> }	53.8	83.4	219



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Transcription factors						
MJ0941	601867	600923	putative transcription initiation factor IIIC {Saccharomyces cerevisiae}	20.1	44.1	945
MJ1045	490363	489848	putative transcription termination-antitermination factor nusA {Methanococcus vannielii}	47.9	73.7	516
MJ0372	1134509	1134123	putative transcription termination-antitermination factor nusG {Homo sapiens}	38.6	63.8	387
MJ0507	1024170	1024631	TATA-binding transcription initiation factor {Thermococcus celer}	51.4	74.0	462
MJ0782	766586	768592	transcription initiation factor IIB {Pyrococcus woesei}	63.8	77.6	2007
MJ1148	384277	384567	transcription-associated protein, ('TFIIS') {Thermococcus celer}	56.4	69.0	291
RNA processing						
MJ0697	849814	849125	fibrillarin-like pre-rRNA processing protein {Methanococcus vannielii}	75.3	88.3	690
Translation						
MJ0160	1308036	1309265	PET112 protein {Saccharomyces cerevisiae}	32.3	53.7	1230
Amino acyl tRNA synthetases						
MJ0564	971657	974149	alanyl-tRNA synthetase (alaRS) {Haemophilus influenzae}	28.0	53.1	2493
MJ0237	1244137	1242641	arginyl-tRNA synthetase {Mycobacterium leprae}	31.3	52.7	1497
MJ1555	1605935	1604679	aspartyl-tRNA synthetase {Pyrococcus sp.}	57.8	75.6	1257
MJ1377	145796	147325	glutamyl-tRNA synthetase {Methanobacterium thermoautotrophicum}	51.7	73.6	1530
MJ0228	1253254	1251524	glycyl-tRNA synthetase {Schizosaccharomyces pombe}	45.8	65.2	1731
MJ1000	543634	542396	histidyl-tRNA synthetase {Streptococcus equisimilis}	35.5	56.3	1239

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MJ0947	591914	594817	isoleucyl-tRNA synthetase {Methanobacterium thermoautotrophicum}	52.1	70.0	2904
MJ0633	912642	910015	leucyl-tRNA synthetase {Saccharomyces cerevisiae}	34.4	54.9	2628
MJ1263	266697	264745	methionyl-tRNA synthetase {Haemophilus influenzae}	35.6	56.0	1953
MJ0487	1041343	1039994	phenylalanyl-tRNA synthetase, subunit alpha {Saccharomyces cerevisiae}	41.0	64.0	1350
MJ1108	423555	425198	phenylalanyl-tRNA synthetase, subunit beta {Saccharomyces cerevisiae}	31.6	55.4	1644
MJ1238	287985	289172	prolyl-tRNA synthetase {Homo sapiens}	39.3	59.5	1188
MJ1197	332116	330257	threonyl-tRNA synthetase {Synechocystis sp.}	29.1	52.1	1860
MJ1415	96418	95369	tryptophanyl-tRNA synthetase {Schizosaccharomyces pombe}	30.5	55.3	1050
MJ0389	1118380	1117616	tyrosyl-tRNA synthetase {Homo sapiens}	39.9	63.7	765
MJ1007	536642	534186	valyl-tRNA synthetase {Bacillus stearothermophilus}	36.1	56.6	2457
<b>Degradation of proteins, peptides, and glycopeptides</b>						
MJ1176	356300	357370	ATP-dependent 26S protease regulatory subunit 4 {Homo sapiens}	51.0	74.1	1071
MJ1494	4302	5123	ATP-dependent 26S protease regulatory subunit 8 {Methanobacterium thermoautotrophicum}	58.6	78.2	822
MJ1417	93716	91932	ATP-dependent protease La {Bacillus brevis}	32.8	54.3	1785
MJ0090	1387867	1386755	collagenase {Porphyromonas gingivalis}	32.6	55.2	1113
MJ1130	400455	401969	O-sialoglycoprotein endopeptidase {Saccharomyces cerevisiae}	50.6	67.9	1515
MJ0651	891988	892842	protease IV {Haemophilus influenzae}	35.0	56.2	855
MJ0591	947601	946861	proteasome, subunit alpha {Methanosarcina thermophila}	57.5	78.8	741

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MJ1237	289440	289967	proteasome, subunit beta {Methanosarcina thermophila}	47.5	68.2	528
MJ0806	742381	743364	xaa-pro dipeptidase {Lactobacillus delbrueckii}	36.1	65.2	984
MJ0996	547987	546635	Zn protease {Haemophilus influenzae}	33.9	55.0	1353
<b>Protein modification</b>						
MJ0814	733804	734793	deoxyhypusine synthase {Homo sapiens}	50.0	70.7	990
MJ1274	253925	254653	diphthine synthase {Saccharomyces cerevisiae}	40.7	61.5	729
MJ0172	1296723	1297175	L-isoaspartyl protein carboxyl methyltransferase {Escherichia coli}	47.6	59.4	453
MJ1329	192979	192098	methionine aminopeptidase {Saccharomyces cerevisiae}	36.2	55.1	882
MJ1530	1630123	1629764	N-terminal acetyltransferase complex, subunit ARD1 {Homo sapiens}	39.7	55.7	360
MJ1591	1573833	1573072	selenium donor protein {Homo sapiens}	34.3	57.1	762
<b>Ribosomal proteins: synthesis and modification</b>						
MJ0509	1022576	1023502	acidic ribosomal protein P0 (L10E) {Methanococcus vannielii}	63.2	82.1	927
MJ0242	1240163	1240228	ribosomal protein HG12 {Catus (cat)}	63.7	81.9	66
MJ1203	325110	325460	ribosomal protein HS6-type {Haloarcula marismortui}	47.0	71.4	351
MJ0510	1021912	1022460	ribosomal protein L1 {Methanococcus vannielii}	64.5	80.3	549
MJ0373	1133926	1133540	ribosomal protein L11 {Sulfolobus solfataricus}	47.2	72.4	387
MJ0508	1023632	1023937	ribosomal protein L12 {Methanococcus vannielii}	72.8	80.9	306
MJ0194	1282568	1282260	ribosomal protein L13 {Haloarcula marismortui}	44.9	66.4	309
MJ0466	1058694	1058452	ribosomal protein L14 {Methanococcus vannielii}	78.8	92.5	243

MJ0657	888216	887977	ribosomal protein L14B {Saccharomyces cerevisiae}	36.4	59.8	240
MJ0477	1052625	1052302	ribosomal protein L15 {Methanococcus vannielii}	62.7	79.5	324
MJ0983	556982	557290	ribosomal protein L15B {Thermoplasma acidophilum}	62.3	78.6	309
MJ0474	1054523	1053939	ribosomal protein L18 {Methanococcus vannielii}	73.3	84.3	585
MJ0473	1054978	1054559	ribosomal protein L19 {Methanococcus vannielii}	67.0	86.4	420
MJ0179	1291786	1291052	ribosomal protein L2 {Methanococcus vannielii}	74.0	87.0	735
MJ0040	1431958	1432260	ribosomal protein L21 {Haloarcula marismortui}	54.5	62.3	303
MJ0460	1061493	1061089	ribosomal protein L22 {Haloarcula marismortui}	40.7	61.7	405
MJ0178	1292097	1291840	ribosomal protein L23 {Methanococcus vannielii}	69.8	91.9	258
MJ0467	1058340	1058062	ribosomal protein L24 {Methanococcus vannielii}	70.5	83.0	279
MJ1201	325929	326078	ribosomal protein L24E {Haloarcula marismortui}	54.6	66.7	150
MJ0462	1060388	1060212	ribosomal protein L29 {Halobacterium halobium}	51.0	69.9	177
MJ0193	1283076	1282705	ribosomal protein L29E {Haloarcula marismortui}	48.7	68.7	372
MJ0176	1293794	1292934	ribosomal protein L3 {Haloarcula marismortui}	45.2	63.9	861
MJ1044	490704	490399	ribosomal protein L30 {Methanococcus vannielii}	63.9	84.1	306
MJ0049	1421907	1422152	ribosomal protein L31 {Nicotiana glutinosa}	40.9	66.2	246
MJ0472	1055464	1055063	ribosomal protein L32 {Methanococcus vannielii}	58.0	77.4	402
MJ0655	889197	888931	ribosomal protein L34 {Aedes albopictus}	36.8	58.3	267
MJ0098	1380525	1380686	ribosomal protein L37 {Leishmania infantum}	50.0	67.4	162

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MJ0593	945958	945683	ribosomal protein L37a {Homo sapiens}	44.6	58.7	276
MJ0177	1292889	1292134	ribosomal protein L4 {human} {Haloarcula marismortui}	49.4	66.3	756
MJ0707	838122	838229	ribosomal protein L40 {Saccharomyces cerevisiae}	57.6	66.7	108
MJ0249	1236729	1236448	ribosomal protein L44 {Haloarcula marismortui}	38.8	58.1	282
MJ0689	854995	855150	ribosomal protein L46 {Sulfolobus solfataricus}	52.0	70.0	156
MJ0469	1057259	1056723	ribosomal protein L5 {Methanococcus vannielii}	72.5	84.5	537
MJ0471	1056071	1055526	ribosomal protein L6 {Methanococcus vannielii}	66.5	82.5	546
MJ0476	1053137	1052745	ribosomal protein L7 {Methanococcus vannielii}	70.3	88.6	393
MJ0595	944670	944473	ribosomal protein LX {Sulfolobus acidocaldarius}	38.9	66.7	198
MJ0322	1172916	1173218	ribosomal protein S10 {Pyrococcus woesei}	67.0	91.0	303
MJ0191	1283956	1283735	ribosomal protein S11 {Haloarcula marismortui}	67.2	80.0	222
MJ1046	489559	489260	ribosomal protein S12 {Methanococcus vannielii}	87.0	96.0	300
MJ0036	1434801	1434352	ribosomal protein S13 {Brugia pahangi.}	49.4	71.0	450
MJ1474	26554	26054	ribosomal protein S15A {Brassica napus}	21.7	48.2	501
MJ0465	1059233	1058883	ribosomal protein S17 {Methanococcus vannielii}	71.6	82.4	351
MJ0245	1238750	1238896	ribosomal protein S17B {Saccharomyces cerevisiae}	55.4	80.9	147
MJ0189	1285220	1284771	ribosomal protein S18 {Arabidopsis thaliana}	42.3	68.5	450
MJ0180	1290861	1290508	ribosomal protein S19 {Haloarcula marismortui}	56.9	73.3	354
MJ0692	853669	854046	ribosomal protein S19S {Ascaris suum}	49.6	67.0	378

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MJ0394	1115064	1115366	ribosomal protein S24 {Haloarcula marismortui}	42.6	64.4	303
MJ0250	1236377	1236192	ribosomal protein S27 {Saccharomyces cerevisiae}	42.6	53.8	186
MJ0393	1115369	1115548	ribosomal protein S27A {Caenorhabditis elegans}	58.4	68.8	180
MJ0461	1061060	1060437	ribosomal protein S3 {Haloarcula marismortui}	49.1	72.1	624
MJ1202	325575	325808	ribosomal protein S33 {Kluyveromyces lactis}	62.1	81.1	234
MJ0980	558761	559252	ribosomal protein S3a {Catharanthus roseus}	29.8	52.1	492
MJ0190	1284710	1284150	ribosomal protein S4 {Sulfolobus acidocaldarius}	51.3	68.4	561
MJ0468	1057935	1057318	ribosomal protein S4E {Methanococcus vannielii}	70.9	84.5	618
MJ0475	1053877	1053275	ribosomal protein S5 {Methanococcus vannielii}	75.7	88.6	603
MJ1260	270075	269683	ribosomal protein S6 {Homo sapiens}	36.2	58.0	393
MJ0620	922671	921799	ribosomal protein S6 modification protein {Haemophilus influenzae}	34.4	57.3	873
MJ1001	542227	541487	ribosomal protein S6 modification protein II {Haemophilus influenzae}	24.8	47.4	741
MJ1047	489046	488627	ribosomal protein S7 {Methanococcus vannielii}	65.8	83.6	420
MJ0470	1056445	1056113	ribosomal protein S8 {Methanococcus vannielii}	71.2	89.2	333
MJ0673	873106	872720	ribosomal protein S8E {Haloarcula marismortui}	50.0	69.7	387
MJ0195	1282118	1281840	ribosomal protein S9 {Haloarcula marismortui}	50.0	75.0	279

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tRNA modification						
MJ0946	595006	596040	N2,N2-dimethylguanosine tRNA methyltransferase {Saccharomyces cerevisiae}	31.6	56.0	1035
MJ1675	1478684	1477755	pseudouridylylate synthase I {Haemophilus influenzae}	33.5	57.2	930
MJ0436	1081116	1082732	queuine tRNA ribosyltransferase {Escherichia coli}	30.4	47.6	1617
Translation factors						
MJ0829	723534	722260	peptide chain release factor, eRF, subunit 1 {Xenopus laevis}	33.0	57.3	1275
MJ1505	1659133	1661085	putative ATP-dependent RNA helicase, eIF-4A family {Saccharomyces cerevisiae}	30.8	51.9	1953
MJ1574	1587062	1588927	putative ATP-dependent RNA helicase, eIF-4A family {Bacillus subtilis}	33.1	56.0	1866
MJ0669	876636	877637	putative ATP-dependent RNA helicase, eIF-4A family {Bacillus subtilis}	44.5	65.8	1002
MJ0495	1035432	1034044	putative translation factor, EF-TU/1 alpha family {Thermus aquaticus}	36.9	55.9	1389
MJ0262	1225060	1221653	putative translation initiation factor, FUN12/bIF-2 family {Saccharomyces cerevisiae}	39.3	61.5	3408
MJ0324	1171724	1172830	translation elongation factor, EF-1 alpha {Methanococcus vannielii}	78.9	90.8	1107
MJ1048	488471	486336	translation elongation factor, EF-2 {Methanococcus vannielii}	74.8	88.5	2136
MJ0445	1073262	1073483	translation initiation factor, eIF-1A {Thermoplasma acidophilum}	52.8	70.3	222
MJ0117	1357516	1358196	translation initiation factor, eIF-2, subunit alpha {Saccharomyces cerevisiae}	32.2	56.5	681
MJ0097	1380885	1381313	translation initiation factor, eIF-2, subunit beta {Drosophila melanogaster}	32.1	60.4	429
MJ1261	269396	268164	translation initiation factor, eIF-2, subunit gamma {Homo sapiens}	52.6	71.9	1233
MJ0454	1066217	1067065	translation initiation factor, eIF-2B, subunit alpha {Saccharomyces cerevisiae}	37.9	56.4	849

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MJ0122	1353264	1354127	translation initiation factor, eIF-2B, subunit delta {Mus musculus}	29.4	54.6	864
MJ1228	300895	301236	translation initiation factor, eIF-5a {Sulfolobus acidocaldarius}	50.0	69.7	342
<b>Transport and binding proteins</b>						
MJ0719	818577	820289	ABC transporter ATP-binding protein {Saccharomyces cerevisiae}	49.6	66.9	1713
MJ1023	518606	517821	ABC transporter ATP-binding protein {Bacillus firmus}	49.2	72.4	786
MJ1572	1590114	1589518	ABC transporter ATP-binding protein {Mycoplasma genitalium}	50.0	87.5	597
MJ0035	1435236	1435829	ABC transporter subunit {Cyanelle Cyanophora}	33.9	58.1	594
MJ1508	1656015	1655446	ABC transporter, probable ATP-binding subunit {Haemophilus influenzae}	45.7	68.3	570
MJ1332	189987	191117	GTP-binding protein {Saccharomyces cerevisiae}	38.7	59.8	1131
MJ1326	196392	195292	GTP-binding protein {Schizosaccharomyces pombe}	51.4	71.5	1101
MJ1408	103449	102430	GTP-binding protein, GTP1/OBG-family {Saccharomyces cerevisiae}	30.5	58.4	1020
MJ1464	39865	38858	hypothetical GTP-binding protein (SP:P40010) {Saccharomyces cerevisiae}	32.0	55.5	1008
MJ1033	507274	506324	magnesium and cobalt transport protein {Haemophilus influenzae}	42.2	57.9	951
MJ0091	1386551	1385751	Na+/Ca+ exchanger protein {Escherichia coli}	32.3	58.6	801
MJ0283	1204330	1203563	nucleotide-binding protein {Homo sapiens}	47.5	68.0	768



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Amino acids, peptides and amines						
MJ0609	933328	934587	amino acid transporter {Arabidopsis thaliana}	21.9	48.7	1260
MJ1343	181359	182519	ammonium transport protein AMT1 {Arabidopsis thaliana}	35.6	53.3	1161
MJ0058	1413598	1414770	ammonium transporter {Escherichia coli}	34.2	52.2	1173
MJ1269	258901	257993	branched-chain amino acid transport protein livH {Escherichia coli}	30.8	54.6	909
MJ1266	261404	260577	branched-chain amino acid transport protein livJ {Escherichia coli}	28.8	55.2	828
MJ1270	257896	256934	branched-chain amino acid transport protein livM {Escherichia coli}	28.7	52.2	963
MJ1196	332430	333311	cationic amino acid transporter MCAT-2 {Mus musculus}	24.6	50.6	882
MJ0304	1185908	1186333	ferrityochelin binding protein {Pseudomonas aeruginosa}	55.6	74.7	426
MJ0796	752786	752118	glutamine transport ATP-binding protein Q {Escherichia coli}	47.9	67.2	669
MJ1267	260465	259707	high-affinity branched-chain amino acid transport ATP-binding protein {Pseudomonas aeruginosa}	34.2	60.8	759
MJ1268	259458	258973	high-affinity branched-chain amino acid transport ATP-binding protein {Salmonella typhimurium}	40.4	68.6	486
Anions						
MJ0412	1099862	1100608	nitrate transport ATP-binding protein {Synechococcus sp}	44.6	70.1	747
MJ0413	1099077	1099826	nitrate transport permease protein {Synechococcus sp}	34.2	59.4	750
MJ1012	529685	530431	phosphate transport system ATP-binding protein {Escherichia coli}	60.9	80.7	747
MJ1013	528941	529642	phosphate transport system permease protein A {Haemophilus influenzae}	39.6	60.5	702
MJ1014	528397	528810	phosphate transport system permease protein C {Haemophilus influenzae}	40.0	66.5	414

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MJ1009	532458	533165	phosphate transport system regulatory protein {Escherichia coli}	28.5	54.6	708
MJ1015	526871	527698	phosphate-binding protein {Xanthomonas oryzae}	45.8	60.2	828
<b>Carbohydrates, organic alcohols, and acids</b>						
MJ0576	960439	959399	malic acid transport protein {Schizosaccharomyces pombe}	23.8	47.9	1041
MJ0762	786703	787524	malic acid transport protein {Schizosaccharomyces pombe}	26.5	49.3	822
MJ0121	1354728	1355291	SN-glycerol-3-phosphate transport ATP-binding protein {Escherichia coli}	33.4	51.7	564
MJ1319	206861	205926	sodium-dependent noradrenaline transporter {Haemophilus influenzae}	37.8	61.0	936
<b>Cations</b>						
MJ1088	444480	445223	cobalt transport ATP-binding protein O {Salmonella typhimurium}	46.1	66.6	744
MJ1090	443372	443527	cobalt transport protein N {Salmonella typhimurium}	59.1	79.6	156
MJ1089	443778	444374	cobalt transport protein Q {Salmonella typhimurium}	28.9	55.6	597
MJ0089	1388820	1388059	ferric enterobactin transport ATP-binding protein {Escherichia coli}	33.1	59.6	762
MJ0873	674824	674123	ferric enterobactin transport ATP-binding protein {Escherichia coli}	31.5	60.3	702
MJ0566	967842	969857	ferrous iron transport protein B {Escherichia coli}	35.8	61.2	2016
MJ0877	670239	670442	hemin permease {Haemophilus influenzae}	27.9	62.3	204
MJ0087	1390284	1389385	hemin permease {Yersinia enterocolitica}	40.6	67.7	900
MJ0085	1392668	1391613	iron transport system binding protein {Bacillus subtilis}	32.9	53.3	1056
MJ0876	670677	671498	iron(III) dicitrate transport system permease protein {Escherichia coli}	30.8	52.8	822
MJ1441	64080	60403	magnesium chelate subunit {Arabidopsis thaliana}	35.3	57.3	3678

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MJ0911	628932	629972	magnesium-chelatase subunit {Euglena gracilis}	54.9	73.4	1041
MJ1275	253661	252597	NA(+)/H(+) antiporter {Enterococcus hirae}	29.8	59.9	1065
MJ0672	873748	874665	Na <sup>+</sup> transporter {Haemophilus influenzae}	39.3	63.1	918
MJ1231	297233	298873	oxaloacetate decarboxylase, alpha subunit {Salmonella typhimurium}	52.0	68.7	1641
MJ1357	164247	165065	putative potassium channel protein {Bacillus cereus}	42.9	66.7	819
MJ1367	154669	155559	sulfate permease (cysA) {Synechococcus sp}	38.5	64.5	891
MJ1368	153995	154666	sulfate/thiosulfate transport protein {Escherichia coli}	30.9	59.4	672
MJ1485	16909	15713	TRK system potassium uptake protein {Escherichia coli}	29.5	58.5	1197
MJ1105	426702	427217	TRK system potassium uptake protein A {Methanosarcina mazei}	39.3	57.6	516
<b>Other</b>						
MJ1142	390844	389885	arsenical pump-driving ATPase {Escherichia coli}	34.7	55.9	960
MJ0822	727897	729522	ATPase, vanadate-sensitive {Methanococcus voltae}	48.1	69.0	1626
MJ0718	820399	821523	chromate resistance protein A {Alcaligenes eutrophus}	27.9	52.4	1125
MJ1226	304219	301988	H <sup>+</sup> -transporting ATPase {Arabidopsis thaliana}	45.1	63.7	2232
MJ1560	1600958	1601974	quinolone resistance norA protein {Staphylococcus aureus}	28.8	51.1	1017

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Other categories						
MJ1365	157333	156458	pheromone shutdown protein {Enterococcus faecalis}	31.2	57.2	876
MJECL24	28069	28845	SOJ protein {Bacillus subtilis}	34.0	62.1	776
Drug and analog sensitivity						
MJ1538	1621434	1620691	K. lactis toxin sensitivity protein KTI12 {Saccharomyces cerevisiae}	28.4	48.8	744
MJ0102	1375563	1375859	phenylacrylic acid decarboxylase {Saccharomyces cerevisiae}	50.0	74.0	297
Phage-related functions and prophages						
MJ0630	915023	914598	sodium-dependent phosphate transporter {Cricetulus griseus}	32.6	60.8	426
Transposon-related functions						
MJ0367	1138754	1138080	integrase {Weeksella zoohelcum}	30.9	54.4	675
MJ0017	1455555	1454946	transposase {Bacillus thuringiensis}	29.5	55.0	610
Other						
MJ1064	466505	467095	acetyltransferase {Escherichia coli}	47.0	62.4	591
MJ1612	1549430	1548297	BcpC phosphonopyruvate decarboxylase {Streptomyces hygroscopicus}	31.1	48.9	1134
MJ0677	868213	869160	ethylene-inducible protein homolog {Hevea brasiliensis}	68.3	81.0	948
MJ0534	1003199	1002072	flavoprotein {Methanobacterium thermoautotrophicum}	34.6	57.2	1128
MJ0748	797504	798673	flavoprotein {Methanobacterium thermoautotrophicum}	67.0	82.6	1170
MJ0256	1230191	1229760	fom2 phosphonopyruvate decarboxylase {Streptomyces wedmorensis}	36.7	58.5	432
MJ1682	1472535	1473320	heat shock protein X {Haemophilus influenzae}	30.4	55.5	786

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MJ0866	682753	682367	HIT protein, member of the HIT-family {Saccharomyces cerevisiae}	39.4	64.8	387
MJ0294	1193529	1195817	large helicase related protein, LHR {Escherichia coli}	31.4	53.6	2289
MJ0010	1460660	1459497	phosphonopyruvate decarboxylase {Streptomyces hygroscopicus}	28.0	47.2	1164
MJ0734	805855	806439	rubrerythrin {Clostridium perfringens}	48.9	69.2	585
MJ0559	978287	977490	surE survival protein {Escherichia coli}	34.7	55.6	798
MJ1100	431754	430489	urease operon protein {Mycobacterium leprae}	33.2	55.0	1266
MJ0543	990687	991100	Wilm's tumor suppressor homolog {Arabidopsis thaliana}	45.6	64.9	414
MJ0765	784011	785549	[6Fe-6S] prismane-containing protein {Desulfovibrio desulfuricans}	60.2	72.8	1539
<b>Hypothetical</b>						
MJ0458	1063165	1062518	hypothetical protein {Sulfolobus acidocaldarius}	-0.9	-0.9	648
MJ0483	1047280	1048250	hypothetical protein {Saccharomyces cerevisiae}	27.7	48.7	971
MJ0920	620866	621357	hypothetical protein {Mycoplasma genitalium}	28.3	51.3	492
MJ0443	1074680	1075348	hypothetical protein {Saccharomyces cerevisiae}	27.8	52.8	669
MJ0144	1330246	1330962	hypothetical protein {Methanobacterium thermoautotrophicum}	33.4	58.6	717
MJ0044	1426552	1427241	hypothetical protein (GP:D38561_6) {Streptomyces wedmorensis}	24.1	49.8	690
MJ0868	680710	681000	hypothetical protein (GP:D63999_31) {Synechocystis sp.}	42.2	65.0	291
MJ1502	1662923	1663714	hypothetical protein (GP:D64001_24) {Synechocystis sp.}	36.4	60.1	792
MJ1129	402152	402382	hypothetical protein (GP:D64001_53) {Synechocystis sp.}	37.5	57.9	231
MJ0057	1414899	1416176	hypothetical protein (GP:D64003_36) {Synechocystis sp.}	28.4	53.2	1278

MJ1335	187757	187593	hypothetical protein (GP:D64004_11) {Synechocystis sp.}	46.2	63.5	165
MJ0640	902502	903458	hypothetical protein (GP:D64005_53) {Synechocystis sp.}	33.9	58.8	957
MJ1347	177726	177280	hypothetical protein (GP:D64006_36) {Synechocystis sp.}	32.1	58.6	447
MJ0392	1116428	1115556	hypothetical protein (GP:D64006_95) {Synechocystis sp.}	29.1	54.3	873
MJ0590	950234	948222	hypothetical protein (GP:D64044_18) {Escherichia coli}	30.6	52.6	2013
MJ1178	355642	355956	hypothetical protein (GP:L47709_14) {Bacillus subtilis}	27.1	55.3	315
MJ0438	1080099	1079128	hypothetical protein (GP:L47838_15) {Bacillus subtilis}	29.6	55.8	972
MJ0644	898810	898223	hypothetical protein (GP:M18279_1) {Pseudomonas sp.}	28.3	53.4	588
MJ0828	723763	723668	hypothetical protein (GP:M35130_5) {M71467 M71468}	58.1	87.1	96
MJ1526	1632280	1632810	hypothetical protein (GP:M36534_1) {Methanobrevibacter smithii}	42.6	66.5	531
MJ0888	652964	653473	hypothetical protein (GP:U00011_3) {Mycobacterium leprae}	29.5	51.4	510
MJ0729	809665	809321	hypothetical protein (GP:U18744_1) {Bacillus firmus}	29.4	56.9	345
MJ0787	761402	760077	hypothetical protein (GP:U19363_11) {Methanobacterium thermoautotrophicum}	49.9	71.9	1326
MJ0693	852445	853059	hypothetical protein (GP:U19363_2) {Methanobacterium thermoautotrophicum}	42.8	61.9	615
MJ0489	1039414	1038686	hypothetical protein (GP:U19363_4) {Methanobacterium thermoautotrophicum}	41.3	57.5	729
MJ0446	1072662	1071784	hypothetical protein (GP:U19363_5) {Methanobacterium thermoautotrophicum}	29.8	50.7	879
MJ0076	1400741	1400403	hypothetical protein (GP:U19364_10) {Methanobacterium thermoautotrophicum}	25.3	56.1	339
MJ0034	1435995	1436921	hypothetical protein (GP:U19364_2) {Methanobacterium thermoautotrophicum}	23.9	49.7	927

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MJ1251	277892	277392	hypothetical protein (GP:U19364_4) {Methanobacterium thermoautotrophicum}	37.8	61.0	501
MJ0927	615224	615694	hypothetical protein (GP:U19364_6) {Methanobacterium thermoautotrophicum}	37.9	57.2	471
MJ0785	763999	762923	hypothetical protein (GP:U19364_8) {Methanobacterium thermoautotrophicum}	57.5	76.6	1077
MJ0746	799630	799935	hypothetical protein (GP:U21086_2) {Methanobacterium thermoautotrophicum}	60.3	76.4	306
MJ1155	378926	380485	hypothetical protein (GP:U28377_114) {Escherichia coli}	40.0	63.7	1560
MJ0653	890904	890359	hypothetical protein (GP:U31567_2) {Methanopyrus kandleri}	42.2	64.8	546
MJ0532	1003608	1004750	hypothetical protein (GP:U32666_1) {Methanosarcina barkeri}	39.3	59.5	1143
MJ0674	872153	871623	hypothetical protein (GP:X83963_2) {Thermococcus litoralis}	58.3	76.7	531
MJ1552	1608984	1608592	hypothetical protein (GP:X85250_3) {Pyrococcus furiosus}	48.5	68.0	393
MJ0709	837195	835996	hypothetical protein (GP:X91006_2) {Pyrococcus sp.}	25.1	50.5	1200
MJ0226	1255943	1255389	hypothetical protein (GP:Z49569_1) {Saccharomyces cerevisiae}	39.0	60.6	555
MJ1476	25468	24851	hypothetical protein (HI0380) {Haemophilus influenzae}	39.7	62.6	618
MJ0441	1076859	1076125	hypothetical protein (HI0902) {Haemophilus influenzae}	29.2	51.1	735
MJ1372	151434	150760	hypothetical protein (HI0920) {Haemophilus influenzae}	46.7	67.5	675
MJ0931	611416	610298	hypothetical protein (MG372) {Mycoplasma genitalium}	34.9	59.9	1119
MJ0861	687240	688532	hypothetical protein (MG423) {Mycoplasma genitalium}	33.9	53.9	1293
MJ1252	277977	278609	hypothetical protein (PIR:B48653) {Lactococcus lactis}	32.5	47.2	633
MJ0279	1206983	1206147	hypothetical protein (PIR:S01072) {Desulfurococcus mobilis}	29.2	53.4	837
MJ0299	1189620	1190600	hypothetical protein (PIR:S11602) {Thermoplasma acidophilum}	62.1	76.6	981

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MJ1208	320842	319766	hypothetical protein (PIR:S21569) {Methanobacterium thermoautotrophicum}	55.4	74.8	1077
MJ1533	1625982	1627727	hypothetical protein (PIR:S28724) {Methanococcus vannielii}	67.3	83.3	1746
MJ0323	1172727	1172257	hypothetical protein (PIR:S38467) {Desulfurococcus mobilis}	60.7	71.7	471
MJ1162	368773	369060	hypothetical protein (PIR:S41581) {Methanothermus fervidus}	48.3	67.9	288
MJ0922	619284	619598	hypothetical protein (PIR:S41583) {Methanothermus fervidus}	48.6	73.4	315
MJ0867	681124	682371	hypothetical protein (PIR:S49379) {Pseudomonas aeruginosa}	28.7	55.2	1248
MJ0047	1423924	1424988	hypothetical protein (PIR:S51413) {Saccharomyces cerevisiae}	26.9	49.9	1065
MJ1236	290570	292111	hypothetical protein (PIR:S51413) {Saccharomyces cerevisiae}	33.9	54.6	1542
MJ0162	1306782	1305562	hypothetical protein (PIR:S51413) {Saccharomyces cerevisiae}	32.4	56.4	1221
MJ0928	614493	614957	hypothetical protein (PIR:S51868) {Saccharomyces cerevisiae}	38.4	61.7	465
MJ1625	1535098	1533113	hypothetical protein (PIR:S52522) {Saccharomyces cerevisiae}	27.6	50.4	1986
MJ0862	686185	687054	hypothetical protein (PIR:S52979) {Erwinia herbicola}	35.5	59.2	870
MJ1432	69872	69453	hypothetical protein (PIR:S53543) {Saccharomyces cerevisiae}	38.5	66.0	420
MJ0710	835912	834914	hypothetical protein (SP:P05409) {Methanococcus thermolithotrophicus}	59.2	79.9	999
MJ0170	1299322	1300185	hypothetical protein (SP:P11666) {Escherichia coli}	30.1	54.8	864
MJ1593	1571988	1571740	hypothetical protein (SP:P12049) {Bacillus subtilis}	40.3	69.6	249
MJ0463	1060127	1059819	hypothetical protein (SP:P14021) {Methanococcus vannielii}	78.5	92.2	309
MJ0464	1059719	1059435	hypothetical protein (SP:P14022) {Methanococcus vannielii}	58.8	79.4	285
MJ0136	1340892	1340105	hypothetical protein (SP:P14027) {Methanococcus vannielii}	63.4	87.8	788



MJ0388	1118696	1119244	hypothetical protein (SP:P15886) {Methanococcus vannielii}	46.9	66.3	549
MJ1225	305183	304425	hypothetical protein (SP:P15889) {Thermophilum pendens}	24.1	53.9	759
MJ1133	398771	397509	hypothetical protein (SP:P22349) {Methanobrevibacter smithii}	45.9	67.4	1263
MJ1273	255725	254676	hypothetical protein (SP:P25125) {Thermus aquaticus}	41.4	60.2	1050
MJ1426	76255	75812	hypothetical protein (SP:P25768) {Methanobacterium ivanovii}	47.3	69.3	444
MJ0549	986782	986360	hypothetical protein (SP:P28910) {Escherichia coli}	33.9	59.3	423
MJ0982	557497	558078	hypothetical protein (SP:P29202) {Haloarcula marismortui}	55.9	75.4	582
MJ0990	552446	552658	hypothetical protein (SP:P31065) {Escherichia coli}	39.2	62.4	213
MJ0326	1170026	1168809	hypothetical protein (SP:P31466) {Escherichia coli}	45.6	71.7	1218
MJ0812	736053	736679	hypothetical protein (SP:P31473) {Escherichia coli}	25.8	54.3	627
MJ0079	1398567	1399694	hypothetical protein (SP:P31473) {Escherichia coli}	38.0	63.3	1128
MJ1586	1578078	1576645	hypothetical protein (SP:P31806) {Escherichia coli}	32.4	52.1	1434
MJ1124	409920	406336	hypothetical protein (SP:P32639) {Saccharomyces cerevisiae}	26.9	51.5	3585
MJ1081	451124	450726	hypothetical protein (SP:P32698) {Escherichia coli}	38.2	62.8	399
MJ1413	97390	97629	hypothetical protein (SP:P33382) {Listeria monocytogenes}	40.0	60.0	240
MJ1170	362086	361820	hypothetical protein (SP:P33382) {Listeria monocytogenes}	42.2	63.9	267
MJ0051	1419978	1419670	hypothetical protein (SP:P34222) {Saccharomyces cerevisiae}	38.5	55.8	309
MJ1523	1636316	1635945	hypothetical protein (SP:P37002) {Escherichia coli}	43.0	65.0	372

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MJ0608	934974	935750	hypothetical protein (SP:P37487) {Bacillus subtilis}	44.3	71.4	777
MJ1661	1493414	1493809	hypothetical protein (SP:P37528) {Bacillus subtilis}	47.0	72.6	396
MJ1582	1580646	1579909	hypothetical protein (SP:P37545) {Bacillus subtilis}	35.4	60.6	738
MJ1375	148221	149408	hypothetical protein (SP:P37555) {Bacillus subtilis}	25.0	48.6	1188
MJ0231	1249786	1250814	hypothetical protein (SP:P37869) {Bacillus subtilis}	40.0	44.0	1029
MJ0882	664582	663910	hypothetical protein (SP:P37872) {Bacillus subtilis}	44.0	68.7	673
MJ0043	1429606	1427252	hypothetical protein (SP:P38423) {Bacillus subtilis}	45.5	58.4	2355
MJ0048	1422159	1422842	hypothetical protein (SP:P38619) {Sulfolobus acidocaldarius}	36.6	59.1	684
MJ0989	552670	553011	hypothetical protein (SP:P39164) {Escherichia coli}	29.0	51.8	342
MJ1115	415733	416479	hypothetical protein (SP:P39364) {Escherichia coli}	27.1	48.3	747
MJ1649	1506277	1507068	hypothetical protein (SP:P39587) {Bacillus subtilis}	28.9	48.5	792
MJ0577	959388	958903	hypothetical protein (SP:P42297) {Bacillus subtilis}	31.6	56.4	486
MJ0531	1004977	1004759	hypothetical protein (SP:P42297) {Bacillus subtilis}	43.3	68.7	219
MJ1247	282030	281677	hypothetical protein (SP:P42404) {Bacillus subtilis}	38.4	60.0	354
MJ0486	1041905	1042681	hypothetical protein (SP:P45476) {Escherichia coli}	30.6	55.7	777
MJ0449	1070080	1069565	hypothetical protein (SP:P46348) {Bacillus subtilis}	31.8	60.7	516
MJ0682	861537	864374	hypothetical protein (SP:P46850) {Escherichia coli}	33.4	53.9	2838
MJ1677	1476726	1476376	hypothetical protein (SP:P46851) {Escherichia coli}	40.3	62.0	351
MJ0588	951068	952243	hypothetical protein GP:L07942_2 {Escherichia coli}	31.1	55.0	1176

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MJ0225	1256840	1256121	hypothetical protein GP:U00014_23 {Mycobacterium leprae}	27.4	49.0	720
MJ0134	1342043	1342792	hypothetical protein GP:U00017_21 {Mycobacterium leprae}	32.2	52.7	750
MJ0376	1130650	1129130	hypothetical protein GP:U29579_58 {Escherichia coli}	30.1	51.5	1521
MJ0028	1443023	1443844	hypothetical protein H11305 {Haemophilus influenzae}	27.0	50.0	822
MJ1136	395844	394486	hypothetical protein Lpg22p (GP:U43281_22) {Saccharomyces cerevisiae}	46.2	63.8	1359
MJ0952	588063	588479	hypothetical protein PIR:S49633 {Saccharomyces cerevisiae}	26.8	55.0	417
MJ0403	1109067	1108276	hypothetical protein PIR:SS5196 {Saccharomyces cerevisiae}	27.6	48.2	792
MJ1031	509420	508506	hypothetical protein SP:P45869 {Bacillus subtilis}	26.8	51.1	915

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Table 2B

MJ0479	1,050,508	1,049,948	adenylate kinase {Methanococcus jannaschii}	100.0%	100.0%	585
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Table 3

MJ0002	4071	3343
MJ0003	4911	5378
MJ0008	10075	10734
MJ0009	10743	11570
MJ0011	12983	13459
MJ0012	13927	13427
MJ0013	14836	14351
MJ0014	15455	14820
MJ0015	15514	15804
MJ0016	16416	15866
MJ0018	17658	19229
MJ0019	21121	19232
MJ0021	22762	23886
MJ0023	25284	25637
MJ0024	26105	25689
MJ0025	27122	26109
MJ0027	28572	28021
MJ0037	38073	38786
MJ0038	39443	38793
MJ0039	39974	39654
MJ0041	41838	40477
MJ0042	42527	41883
MJ0045	46506	45907
MJ0046	47351	46569
MJ0050	52237	51050
MJ0052	53374	52709
MJ0053	54068	53388
MJ0054	55001	54159

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MJ0056	56154	55759
MJ0062	60618	61238
MJ0063	61322	61855
MJ0064	61897	62454
MJ0065	63551	62463
MJ0066	65078	63657
MJ0067	65160	65468
MJ0068	65861	65517
MJ0070	66966	67211
MJ0071	67211	67480
MJ0072	67562	67693
MJ0073	67729	68007
MJ0074	69089	68016
MJ0075	70324	69236
MJ0077	71539	70394
MJ0078	72674	72054
MJ0080	74182	73802
MJ0086	80788	81903
MJ0088	83019	83537
MJ0093	88517	88092
MJ0094	89481	88564
MJ0095	89828	89568
MJ0096	90752	89967
MJ0100	94823	93297
MJ0103	97958	99256
MJ0105	101649	101239
MJ0106	102541	101840
MJ0107	102733	104295
MJ0109	106419	105664
MJ0110	106880	106614

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MJ0114	111874	112782
MJ0115	113249	112785
MJ0116	113931	113257
MJ0119	116397	115726
MJ0120	117070	116372
MJ0123	119524	119195
MJ0125	123378	123031
MJ0126	123685	123392
MJ0127	124034	123672
MJ0128	124341	124048
MJ0129	124487	124996
MJ0131	126783	126475
MJ0133	129427	128609
MJ0137	134976	134119
MJ0138	136566	135121
MJ0139	136616	138244
MJ0140	139150	139539
MJ0141	139529	139825
MJ0142	139797	140237
MJ0145	142991	142188
MJ0146	143409	143203
MJ0147	144813	143701
MJ0149	146003	145830
MJ0150	146069	146587
MJ0154	152143	152589
MJ0157	159807	160085
MJ0158	160155	161276
MJ0159	163046	161430
MJ0163	167378	166818
MJ0164	168614	167430

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MJ0165	169394	168627
MJ0166	170194	169430
MJ0173	175871	176341
MJ0175	178089	177475
MJ0181	182625	181918
MJ0182	183311	182730
MJ0183	183491	183348
MJ0184	183606	183827
MJ0185	183886	184032
MJ0187	185874	185440
MJ0188	186674	185880
MJ0198	191384	192259
MJ0201	193486	193007
MJ0202	193687	194454
MJ0206	198871	198467
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MJ0208	200166	199429
MJ0209	200956	200159
MJ0212	203759	204019
MJ0213	204137	204583
MJ0215	205636	205190
MJ0223	214474	214163
MJ0224	215072	214566
MJ0227	218176	219099
MJ0229	221136	220852
MJ0230	221386	221144
MJ0233	224281	225111
MJ0235	226124	226369
MJ0236	226362	227639
MJ0239	230506	230988



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MJ0240	231618	231094
MJ0241	232062	231628
MJ0243	232563	232318
MJ0248	235142	235651
MJ0251	238728	238288
MJ0252	238849	239487
MJ0255	241359	240607
MJ0257	242764	243696
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MJ0259	245717	245112
MJ0261	247082	246423
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MJ0271	256902	257441
MJ0272	257452	257649
MJ0273	258107	258412
MJ0274	260378	258819
MJ0275	261121	260516
MJ0280	266375	266758
MJ0281	267291	266761
MJ0282	267341	267787
MJ0284	269902	269174
MJ0286	270849	270499
MJ0287	271160	270870
MJ0288	271755	271222
MJ0289	272805	271801
MJ0290	273753	273121
MJ0292	275409	275137
MJ0296	279767	280360
MJ0297	281155	280406

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MJ0298	281290	281739
MJ0301	285101	284220
MJ0303	285971	285558
MJ0305	286594	287778
MJ0306	287997	287818
MJ0308	289084	288386
MJ0310	290609	290268
MJ0311	290981	290652
MJ0312	291845	291228
MJ0314	293767	294369
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MJ0316	295458	294964
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MJ0319	297675	297902
MJ0320	298001	298645
MJ0321	298675	299040
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MJ0329	306607	304760
MJ0330	308266	306620
MJ0331	308670	308266
MJ0332	308995	308678
MJ0333	309670	309410
MJ0334	309816	310112
MJ0335	310179	310919
MJ0336	310932	311288
MJ0337	311299	312084
MJ0338	312100	312402
MJ0339	312374	312694

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MJ0340	312697	313398
MJ0341	313411	313770
MJ0342	313918	314286
MJ0343	314270	316807
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MJ0345	317314	318264
MJ0346	318277	318579
MJ0347	318593	319045
MJ0348	319620	321995
MJ0349	322367	322053
MJ0350	322681	322418
MJ0351	323154	322705
MJ0352	323901	323185
MJ0353	324142	323891
MJ0354	324296	324123
MJ0355	324661	324374
MJ0356	324957	324697
MJ0357	326407	325943
MJ0358	326796	326413
MJ0359	327449	326808
MJ0360	328174	327770
MJ0361	329502	329182
MJ0362	329659	329847
MJ0364	332163	332495
MJ0365	332503	333030
MJ0366	333033	333308
MJ0368	334581	334886
MJ0369	336040	334934
MJ0371	337418	337639
MJ0374	339873	338884

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MJ0375	339920	340681
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MJ0385	350252	351304
MJ0386	351648	351307
MJ0390	355149	354760
MJ0395	357787	357314
MJ0398	359111	359923
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MJ0421	379640	380719

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MJ0482	423793	423074
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MJ0494	436499	436891

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MJ0545	483451	482711
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MJ0560	495301	494891
MJ0562	496903	496691
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MJ0572	509860	510117
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MJ0574	510865	511143
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MJ0581	515692	515937
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MJ0584	516563	517657
MJ0585	517680	518294
MJ0586	518563	519057
MJ0587	519994	519536
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MJ0594	526886	527392
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MJ0597	528539	529612
MJ0599	530524	531120
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MJ0604	535443	535144
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MJ0606	536194	535922
MJ0607	536435	536199
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MJ0614	545444	545061
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MJ0619	549378	547861
MJ0621	551088	550573
MJ0623	552787	553362
MJ0625	553606	554613
MJ0626	554709	555335
MJ0627	555369	555719
MJ0628	555715	556203
MJ0629	556208	556849
MJ0632	558292	559380
MJ0634	562682	564565
MJ0635	564797	565636
MJ0638	568586	567912
MJ0639	568870	568586
MJ0642	571462	572451
MJ0645	574498	574743
MJ0646	574757	575248
MJ0647	575457	575296
MJ0648	575881	575441
MJ0650	577458	579521
MJ0652	580869	580471
MJ0659	585626	586039
MJ0660	586366	586136
MJ0661	587014	586496



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MJ0662	587657	587007
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MJ0665	590629	590180
MJ0668	594556	594314
MJ0670	596945	595887
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MJ0678	605240	604263
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MJ0686	615407	613668
MJ0687	616482	615478
MJ0688	616670	617110
MJ0690	617965	617375
MJ0691	618300	617974
MJ0694	620244	621365
MJ0695	621809	621486
MJ0696	622409	621933
MJ0699	625837	624698
MJ0700	625851	626822
MJ0701	626831	628063
MJ0702	628050	629831
MJ0703	629859	630536
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MJ0708	634868	634425
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MJ0717	650815	650459
MJ0724	657809	657189

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MJ0730	663605	663048
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MJ0745	672208	671675
MJ0747	673416	672961
MJ0749	675903	675151
MJ0750	676710	675997
MJ0751	677628	676795
MJ0752	677942	677715
MJ0753	678766	678146
MJ0754	679347	678775
MJ0755	680644	679619
MJ0756	681296	680889
MJ0757	682155	681424
MJ0758	682653	682213
MJ0759	683029	682700
MJ0760	683871	683047
MJ0761	684833	684072
MJ0763	686251	685889
MJ0764	686611	686264
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MJECL13	10678	12483
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MJECL38	47997	47197
MJECL39	49387	48329
MJECL41	53908	52613
MJECL43	57371	56187
MJECL44	58339	57341

Table 4

Genes of <i>M. jannaschii</i> that contain inteins.		
Gene No.	Putative identification	No. of inteins
MJ0043	Hypothetical protein ( <i>Bacillus subtilis</i> )	1
MJ0262	Putative translation initiation factor, FUN12/IF-2 family	1
MJ0542	Phosphoenolpyruvate synthase	1
MJ0682	Hypothetical protein ( <i>Escherichia coli</i> )	1
MJ0782	Transcription initiation factor IIB	1
MJ0832	Anaerobic ribonucleoside-triphosphate reductase	2
MJ0885	DNA-dependent DNA polymerase, family B	2
MJ1042	DNA-dependent RNA polymerase, subunit A'	1
MJ1043	DNA-dependent RNA polymerase, subunit A''	1
MJ1054	UDP-glucose dehydrogenase	1
MJ1124	Hypothetical protein ( <i>Saccharomyces cerevisiae</i> )	1
MJ1420	Glutamine-fructose-6-phosphate transaminase	1
MJ1422	Replication factor C, 37-kD subunit	3
MJ1512	Reverse gyrase	1

PCT1.WPD

The 1,664,976 *M. jannaschii* circular chromosome (SEQ ID NO:1) has the following sequence:

5 GGATTATTATGCTACTGGTTTTAAATAATTGACTTATCTAACTAAAAGGAGGAATTAA  
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A A C A A T T T T A A T G C T G C C A A A C C A G C C A A T A T T G G A G C T C C A T A A A A A T C T T A C T A C C  
T C C A A T A A T C A A A A C T T T C C A T T T T G C C C T T T G T G G C T A T T G C T A T C T C T C T T C C T T A G  
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T G C C T T T C C A G C A T T C C C A T T A A T A A T A A C C T C T T T T C T T A A T T T T T G T T T A A  
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40 The 58,407 bp *M. jannaschii* large circular extrachromosomal element  
 (SEQ ID NO:2) has the following sequences:

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 45 CATACCAGGAATAAATTTTAATTAATTTAGATATTAATCTCATTATAGGTGTTAAAAATG  
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60 ACTTTATAAACAAATGTCTTAAACTAAAATAAAGTCCCGATTTCAATTAATGTACAA  
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The 16,550 bp *M. jannaschii* small circular extrachromosomal element  
(SEQ ID NO:3) has the following sequence:

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10  CTAATAGATGTTCTTCATCCCCACTGTATAATTTTGGTTGGAGCGGAGCTTTTAAGGAAC
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15  TTTTCTAATCTTTTGTATATTCCCATTTTTTAAAGTGAGGATTATATCCATAATCTCCT
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While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

5           All patents, patent applications and publications recited herein are hereby incorporated by reference.

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***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

5 (a) a nucleotide sequence of an open reading frame depicted in Table 2(a) or 3;

(b) a nucleotide sequence of an open reading frame depicted in Table 2(a) or 3, but minus the codon for the N-terminal methionine residue, if present; and

10 (c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b).

2. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence 100% identical to a sequence in (a), (b) or (c) of claim 1.

15 3. An isolated nucleic acid molecule comprising a polynucleotide that hybridizes under stringent hybridization conditions to the nucleic acid molecule of claim 2.

20 4. An isolated nucleic acid molecule comprising a polynucleotide that encodes the amino acid sequence of an epitope-bearing portion of the *M. jannaschii* protein encoded by an open reading frame depicted in Table 2(a) or 3.

5. A method of making a recombinant vector comprising inserting the isolated nucleic acid molecule of claim 1 into a vector.

6. A recombinant vector produced by the method of claim 5.

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7. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 6 into a host cell.

8. A recombinant host cell produced by the method of claim 7.

5 9. A recombinant method for producing a *M. jannaschii* polypeptide, comprising culturing the recombinant host cell of claim 8 under conditions such that said polypeptide is expressed and recovering said polypeptide.

10 10. An isolated polypeptide having an amino acid sequence at least 95% identical to the amino acid sequence selected from the group consisting of:  
(a) an amino acid sequence encoded by a *M. jannaschii* open reading frame depicted in Table 2(a) or 3; and

(b) an amino acid sequence encoded by a *M. jannaschii* open reading frame depicted in Table 2(a) or 3, but lacking the N-terminal methionine residue.

15 11. An isolated polypeptide, wherein said amino acid sequence is 100% identical to a sequence in (a) or (b) of claim 10.

12. An isolated antibody that binds specifically to the polypeptide of claim 11.

20 13. Computer readable medium having recorded thereon the nucleotide sequence depicted in SEQ ID NO:1, 2, or 3, or a nucleotide sequence at least 99.9% identical thereto.

14. Computer readable medium having recorded thereon the nucleotide sequence of at least one *M. jannaschii* open reading frame depicted in Table 2(a) or 3 or its complement.



15. The computer readable medium of claim 13, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

5 16. The computer readable medium of claim 14, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

17. A computer-based system for identifying fragments of the *M. jannaschii* genome that are homologous to target nucleotide sequences, comprising:

10 (a) a data storage means comprising the nucleotide sequence of SEQ ID NO:1, 2, or 3, or a nucleotide sequence at least 99.9% identical thereto;

(b) a search means for comparing a target sequence to said nucleotide sequence of said data storage means of step (a) to identify a homologous sequence, and

15 (c) a retrieval means for obtaining said homologous sequence of step (b).

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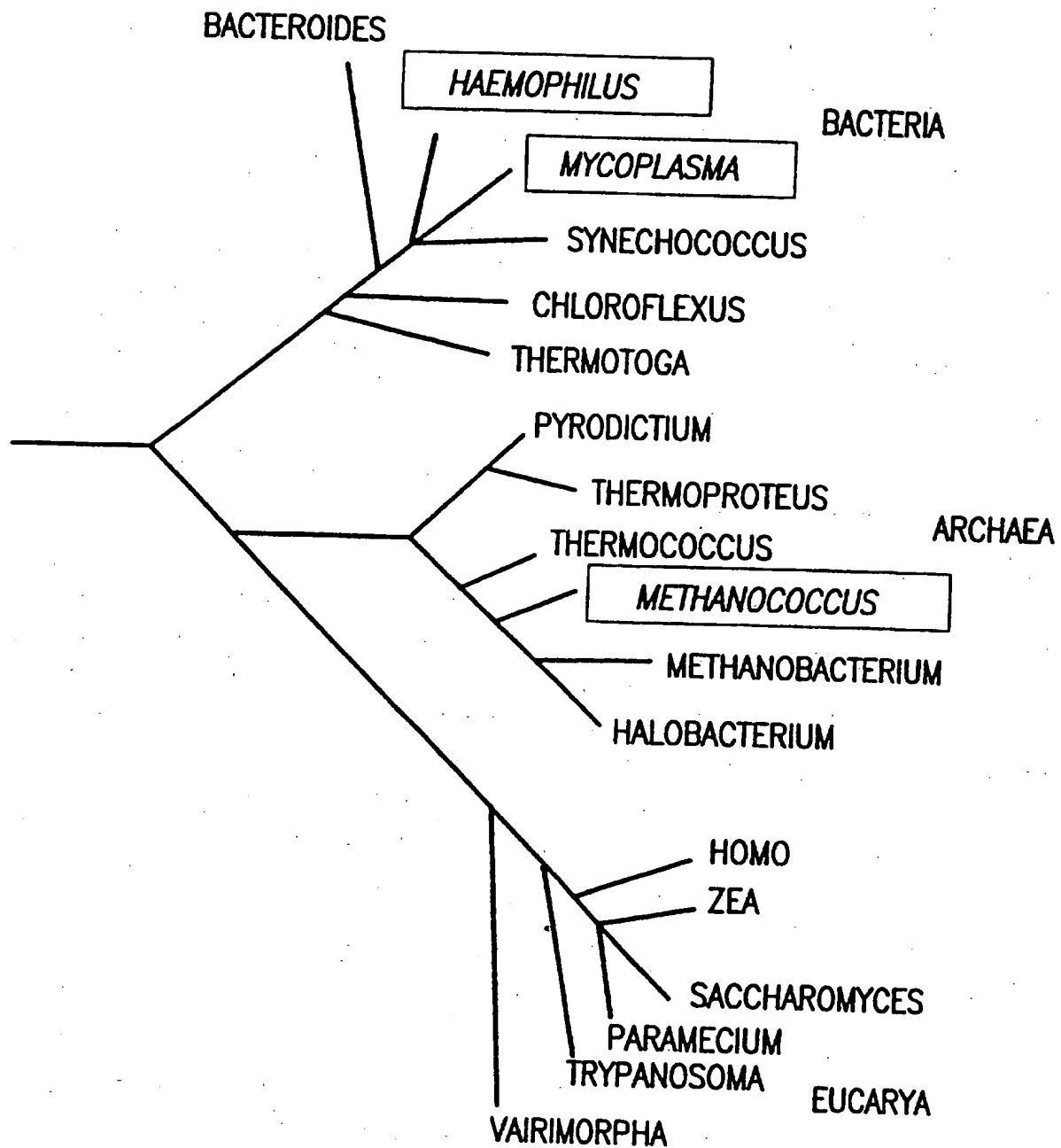


FIG.1

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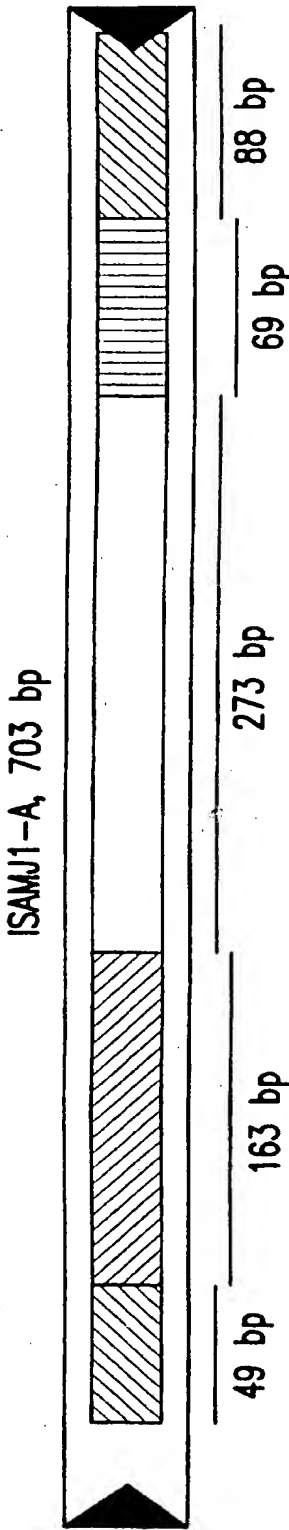


FIG.2A

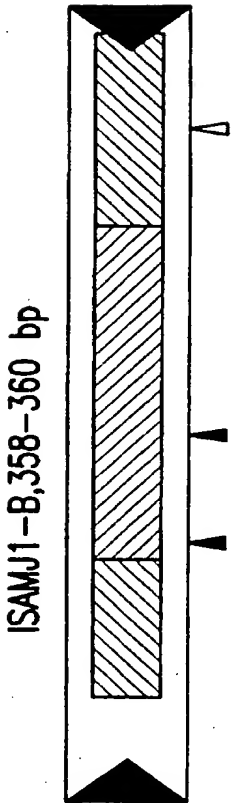


FIG.2B

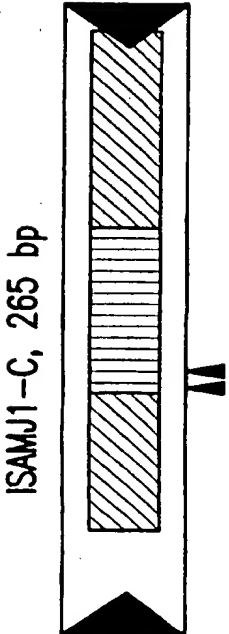


FIG.2C

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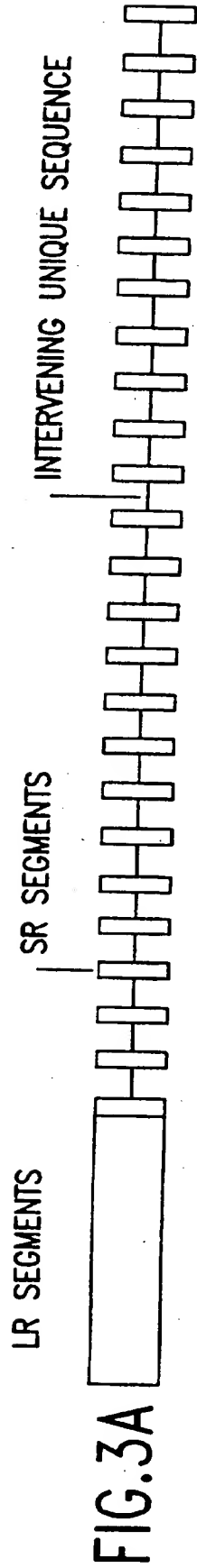


FIG. 3A



FIG. 3B

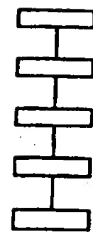


FIG. 3C



FIG. 3D

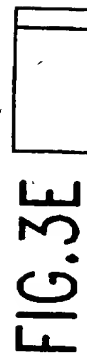


FIG. 3E

SUBSTITUTE SHEET (RULE 26)

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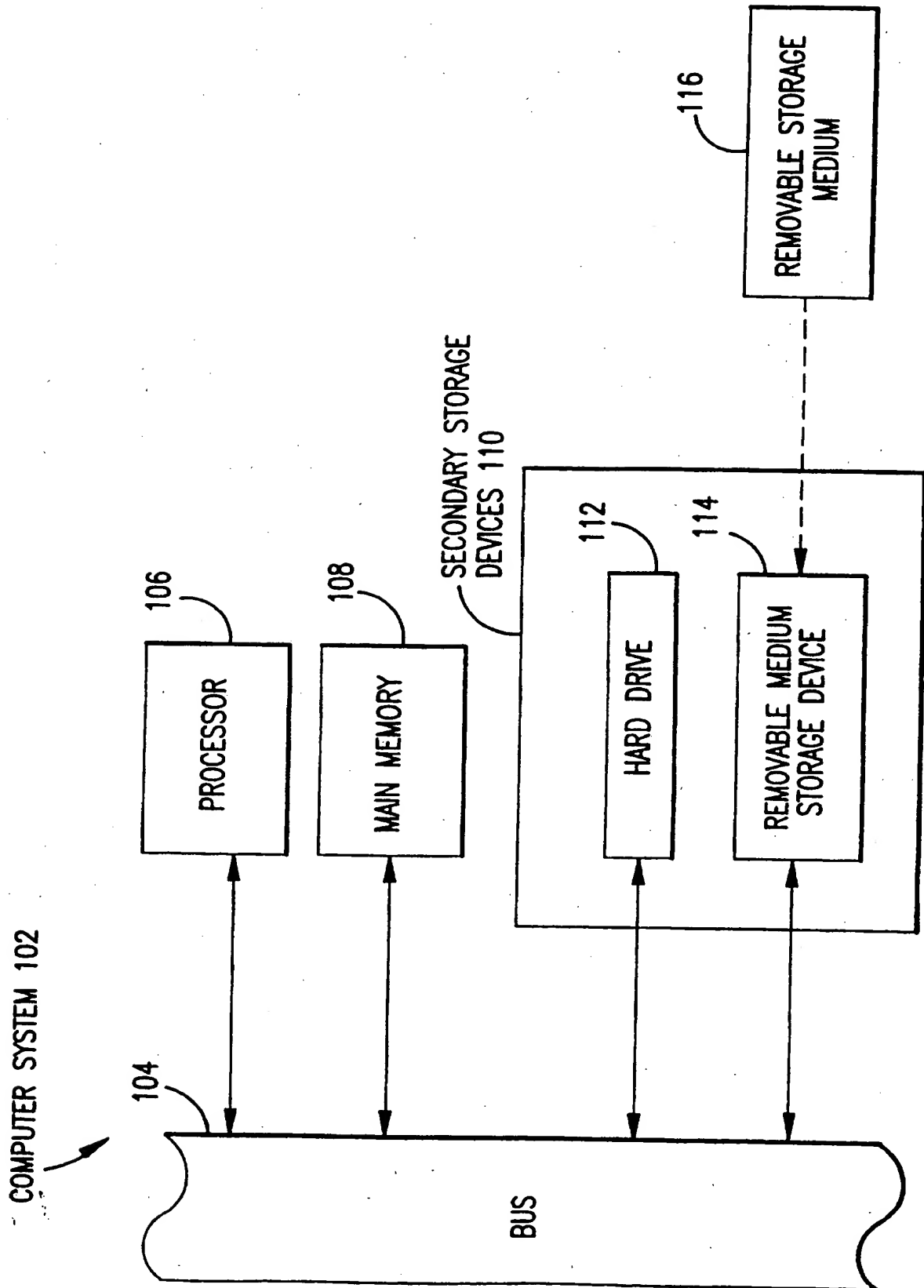


FIG.4





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> A61K 39/00, 39/395, C07H 21/04, C07K 1/00, 14/00, C12N 1/20, 15/00, C12P 21/06, G11B 5/74, 5/82	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 98/07830</b>  <b>(43) International Publication Date:</b> 26 February 1998 (26.02.98)
<b>(21) International Application Number:</b> PCT/US97/14900  <b>(22) International Filing Date:</b> 22 August 1997 (22.08.97)  <b>(30) Priority Data:</b> 60/024,428                      22 August 1996 (22.08.96)                      US  <b>(71) Applicants:</b> THE INSTITUTE FOR GENOMIC RESEARCH [US/US]; 9712 Medical Center Drive, Rockville, MD 20850 (US). THE BOARD OF TRUSTEES OF THE UNIVER- SITY OF ILLINOIS [US/US]; 506 S. Wright Street, Ur- bana, IL 61802 (US). JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; Department of Molec- ular Biology and Genetics, Baltimore, MD 21205 (US).  <b>(72) Inventors:</b> BULT, Carol, J.; Box 525, Bar Harbor, ME 04609 (US). WHITE, Owen, R.; 886 Quince Orchard Boulevard # 202, Gaithersburg, MD 20878 (US). SMITH, Hamilton, O.; 8222 Carrbridge Circle, Baltimore, MD 21204 (US). WOESE, Carl, R.; 806 West Delaware Avenue, Urbana, IL 61801 (US). VENTER, J., Craig; 9708 Medical Center Drive, Rockville, MD 20850 (US).  <b>(74) Agents:</b> STEFFE, Eric, K. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).		<b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 26 March 1998 (26.03.98)
<b>(54) Title:</b> COMPLETE GENOME SEQUENCE OF THE METHANOGENIC ARCHAEON, <i>METHANOCOCCUS JANNASCHII</i>  <b>(57) Abstract</b>  The present application describes the complete 1.66-megabase pair genome sequence of an autotrophic archaeon, <i>Methanococcus jannaschii</i> , and its 58- and 16-kilobase pair extrachromosomal elements. Also described are 1738 predicted protein-coding genes.		

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/14900

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 184.1; 435/69.3, 252.3, 320.1, 325; 530/350; 536/23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CA, EMBASE, WPIDS

terms: Methanococcus, jannaschii, genome, chromosome, dna

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALMOND et al. Complementation of a thr-1 mutation of Escherichia coli by DNA from the extremely thermophilic archaeobacterium Methanococcus jannaschii. Appl. Microbiol. Biotechnol. 1989, Vol. 30, pages 148-152, see entire document.	1-17
Y	FLEISCHMANN et al. Whole-Genome Random Sequencing and Assembly of Haemophilus influenzae Rd. Science. 28 July 1995, Vol. 269, pages 496-512, see entire document.	1-17
Y	US 4,431,739 A (Riggs) 14 February 1984 (14/02/84), see entire document.	5-11
Y	US 4,601,980 A (GOEDDEL ET AL) 22 July 1986 (22/07/86), see entire document.	5-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

15 DECEMBER 1997

Date of mailing of the international search report

10 FEB 1998

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/14900

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEVIER et al. Monoclonal Antibodies in Clinical Immunology. Clinical Chemistry. 1981, Vol. 27, No. 11, pages 1797-1806, see entire document.	12
Y	US 5,518,911 A (ABO ET AL) 21 May 1996 (21/05/96), see entire document, especially column 10, lines 20-30.	13-17

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

**INTERNATIONAL SEARCH REPORT**

**International application No.**

**PCT/US97/14900**

**A. CLASSIFICATION OF SUBJECT MATTER:**

**IPC (6):**

**A61K 39/00, 39/395; C07H 21/04; C07K 1/00, 14/00; C12N 1/20, 15/00; C12P 21/06; G11B 5/74, 5/82**

**A. CLASSIFICATION OF SUBJECT MATTER:**

**US CL :**

**360/131, 135; 424/130.1, 184.1; 435/69.3, 252.3, 320.1, 325; 530/350; 536/23.7**

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